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A STUDY WITH P^{32} OF THE DEPENDENCE OF PHOSPHORUS METABOLISM AND CROP YIELD OF PLANTS ON CONDITIONS OF NITROGEN NUTRITION

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The plastic nature of plant morphogenetic processes ensures growth and development under widely varying conditions. This vitally important characteristic of plants greatly complicates the problem of biologists in studying the effect of nutritional regime employed during the growth period on plant productivity. In solving this problem it is necessary to bear in mind that the mechanism and kinetics of mineral absorption by plants are not critical to an understanding of the role played by minerals in the nutritional cycle.

Physiological studies of phosphorus absorption and transport have been considerably extended by the use of P^{32}. The advantage of this type of approach is fully obvious and has been demonstrated many times. It is also clear, however, that data on incorporation of P^{32} into organic compounds or on transport of P^{32} into various plant organs are not sufficient to permit conclusions as to the effect of phosphorus on plant productivity.

It has long been known that tissues with a given organic content or a given content of economically valuable material may have widely varying levels of phosphorus. In view of this, data on phosphorus absorption are of but little significance, since only the percentage levels of phosphorus in various organs are changed, while plant productivity remains unaltered.

At the same time, studies of this particular aspect of nutrition are of special interest in the establishment of possible new effects on high-yielding plants.

The variable effect of different phosphorus levels on growth and development depends on a number of factors. Age and developmental status of the plant have a great significance in the utilization of phosphorus applied. The previous nutritional regime also plays a very important role. This subject has been considered by Domontovich, a worker in the field of nutrition [1]. The results obtained by Klechkovskii and Bagaev [2] point to the same conclusion.

Among factors determining the utilization of applied phosphorus by the plant, the nitrogen regime is especially important [3-6].

In our studies special attention was devoted to determining the effect of the nitrogen regime and of the plant's nitrogen metabolism on phosphorus absorption and utilization.

Results of studies of plants deprived of phosphorus showed that the nitrogen regime exerts a substantial effect on utilization of seed phosphorus in the formation of organic matter. When ammonium nitrogen was supplied, the plant utilized the phosphorus at its disposal more efficiently. Obviously, the series of reactions required for reduction of an oxidized form of nitrogen is eliminated, and therefore the synthesis of organic compounds proceeds along more efficient pathways. Much attention has been devoted to the role played by phosphorus under conditions in which nitrogen is supplied in the nitrate form. The subject of competition between the nitrate anion and the phosphate anion for absorption has received considerable attention.

Our results have indicated, however, that disturbance of nitrogen metabolism in the root system is of considerably greater importance than anion competition or tissue mineral levels. This has been proven is studies of the dependence of phosphorus absorption on previous nutritional regime.

In an experiment on phosphorus absorption performed in 1948, plants were treated in the following manner: 1) Supplied with all nutrient elements, 2) deprived of phosphorus and nitrogen, 3) deprived of phosphorus. Throughout the day prior to the experiment, plants which had been deprived of phosphorus and nitrogen were exposed to solutions containing nitrogen in either the nitrate or the ammonium form.

Absorption was measured according to the P^{32} content of roots and leaves of various nodes (Fig. 1).

The Curve clearly show that only plants deprived of phosphorus but supplied with nitrogen differ markedly in rate of phosphorus absorption (Curve 1). Phosphorus absorption by plants supplied with ammonium nitrogen (Curve 2) or nitrate nitrogen (Curve 3) just prior to the experiment is not greatly different from that of plants which had developed on a complete nutrient mixture (Curve 4) or on solutions lacking both phosphorus and nitrogen (Curve 5). From this it may be concluded that only in plants in which metabolic cycles between roots and aerial organs have been operative over a prolonged period is the capacity of the roots to release phosphorus to the aerial parts substantially increased.

In this experiment, the nonprotein organic nitrogen content of plants which had developed on complete nutrient mixtures was 0.68%, while that of plants which had developed on solutions lacking phosphorus was 1.96%. Protein nitrogen contents were, respectively, 4.72 and 3.91%.

The effect of nitrogen metabolism of the root system on phosphorus absorption and transport to aerial organs is also determined by plant age. However, the subsequent fate of phosphorus absorbed from the external medium is dependent on age to a still greater degree.

The rate of metabolism of the principal structural phosphorus compounds, nucleoproteins and phosphatides, changes considerably as the plant matures. Reduction in metabolic rate is especially marked in the lower leaves. This occurs to a varying extent in all leaves, however, including the youngest ones. Metabolic turnover of these compounds in roots also declines with age.

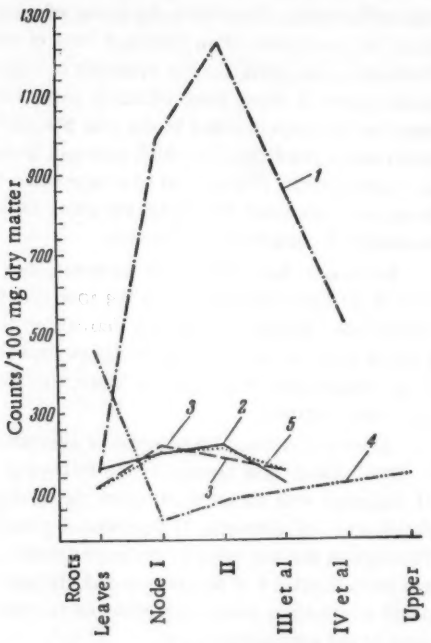

Fig. 1. P^{32} content of various organs of pumpkin under differing experimental conditions. (No. of counts per 100 mg dry matter. Explanation in text.)

Simultaneous studies of the nitrogen regime showed that it is of greater importance than ontogenetic changes in the synthesis and metabolism of nonsoluble phosphorus fractions.

A sharp change in the ratio of phosphorus to nitrogen in the nutrient mixture exerts a particularly strong effect on the plant's nitrogen status. An extreme change of this type is effected by exclusion of phosphorus from a nutrient solution containing nitrogen compounds, especially in the ammonium form. This method, used in our investigations, enabled us to demonstrate that temporary exclusion of phosphorus from the external solution sharply enhances the rate of synthesis of phosphatides and nucleoproteins in various plant organs; the more severe the phosphorus starvation, the greater the increase in rate of synthesis upon addition of phosphorus to the nutrient medium. Differences between plants which were supplied with phosphorus after a temporary deprivation and plants which had developed throughout on a complete medium become greater with age.

A characteristic feature of phosphorus-starved plants is the absence of differences between roots and leaves with respect to rates of synthesis (Table 1).

Data presented in Table 1 reveal very considerable changes in phosphatide and nucleoprotein phosphorus levels, mainly in leaves. In addition, results of studies made over several years show that the character of metabolic processes in the roots has an influence on the metabolism of phosphatides and nucleoproteins in leaves, both as to intensity and quality. In order to throw more light on this subject, however, it was necessary to design an experiment in which phosphorus was applied both to the roots and to excised leaves of plants which had either been deprived of phosphorus or had been grown under normal conditions.

The experiment was performed using pumpkin plants grown for 22 days on nutrient mixtures with and without phosphates. Results obtained showed that the ability of excised leaves to synthesize phosphatides and nucleoproteins does not depend on the nutritional regime to which the plant from which they came had been subjected. This is not true of unexcised leaves. In Table 2, in which data on unexcised leaves are presented, it can be seen that synthesis in plants which had been constantly exposed to a complete nutrient mixture was practically the same regardless of whether phosphorus was supplied through the roots or through the leaves. Plants supplied with phosphorus subsequent to a starvation period behaved otherwise. Rates of synthesis of both fractions were 15-20 times higher where phosphorus was absorbed through the roots. Evidently metabolic processes in roots of phosphorus-starved plants are closely involved in the accumulation of intermediates for nucleoprotein synthesis in leaves. Upon transfer of the starved plants to complete mixtures and rapid acceleration of flow from the roots, and even from the lower nodes, into the assimilating organs, there is a rapid increase in protein phosphorus content.

Results of determinations of nucleoprotein phosphorus in leaves of pumpkin plants deprived of phosphorus lead to the conclusion that evaluation of the significance of the root system in this process is quite complex.

For this experiment, only plants which had been deprived of phosphorus were used. Nucleoprotein content of leaves from various nodes was determined prior to application of phosphorus. As in the previously described experiment, application was made either to the root system or to excised leaves floating in dishes. The experi-

TABLE 1. Synthesis of Nucleoproteins and Phosphatides by Various Organs in Plants of Different Ages under Different Nutritional Regimes (No. of P^{32} counts per 100 mg dry wt. per day)

Organ	Phosphorus compound	10 day-old plants		20 day-old plants	
		C*	−P→P	C*	−P→P
Roots	Phosphatides	304	432	132	360
	Nucleoprotein	1636	1748	816	1330
Cotyledons	Phosphatides	53	304	–	–
	Nucleoprotein	80	286	–	–
Leaves of first node	Phosphatides	175	540	6	344
	Nucleoprotein	207	588	6	338
Leaves of second node	Phosphatides	–	–	7	630
	Nucleoprotein	–	–	16	342
Upper leaves	Phosphatides	–	–	63	772
	Nucleoprotein	–	–	93	940

*C—complete nutrient mixture.

TABLE 2. Content of Organic Nonsoluble Phosphorus of Nucleoproteins and Phosphatides in Leaves from Various Nodes of Pumpkins Exposed to Different Nutritional Regimes (No. of counts per 100 mg dry wt.)

Node	Phosphorus compound	Complete nutrient mixture		−P→+ P	
		→+ P^{32} root absorbed	→+ P^{32} in dishes	→+ P^{32} root absorbed	→+ P^{32} in dishes
3	Organic nonsoluble	126.0	141.0	4500.0	229.0
	Phosphatide	83.4	57.0	2040	101.0
	Nucleoprotein	42.6	84.0	2460	128.0
4	Organic nonsoluble	153.0	173.0	4690	297.0
	Phosphatide	91.4	67.0	2680	131.0
	Nucleoprotein	61.6	97.0	2010	166.0
5	Organic nonsoluble	196.0	209.0	5900	359.0
	Phosphatide	98.0	104.0	3100	175.0
	Nucleoprotein	98.0	105.0	2800	184.0
6	Organic nonsoluble	658.0	691.0	7110	714.0
	Phosphatide	304.0	311.0	3200	306.0
	Nucleoprotein	354.0	380.0	3910	408.0

ment was continued for five days. In addition to nucleoprotein, leaf dry weight was determined at the beginning and end of the experiment. Thus, it was possible to measure not only changes in the total amount, but also those in the relative amount of nucleoprotein. Results of these determinations, which are presented in Table 3, indicate that the total nucleoprotein content of leaves supplied with phosphorus after excision increases considerably. Examination of the data reveals that this increase is due solely to an increase in the organic content of the leaf. There is, however, no relative increase of nucleoprotein. Where leaves remain on the plant, the situation is completely different. The total amount of nucleoprotein phosphorus increases not only because of a certain amount of growth but also because of a substantial increase in this faction relative to total organic content.

To clarify the role of the root system in the synthesis of these phosphorus compounds, still another approach was used which is based on the following assumption.

If the root system plays a considerable role in the synthesis of nucleoprotein, removal of all leaves but one should be reflected in the content and distribution of nucleoprotein phosphorus between this leaf and the root system. An experiment of this type was performed with pumpkin in the summer of 1948. Plants were grown on nutrient mixtures lacking phosphorus for 18 days. During this time five leaves developed. On the day before

TABLE 3. Content of Nucleoprotein Phosphorus in Leaves of the Second and Third Nodes from Plants Grown under Various Nutritional Regimes

Items determined	−P		−P→+ P to excised leaves		−P→+ P to soil	
	2	3	2	3	2	3
Dry weight	0.225	0.341	0.480	0.397	0.329	0.450
Percent of nucleoprotein P	0.046	0.074	0.047	0.077	0.066	0.117
Total nucleoprotein P	0.104	0.260	0.202	0.305	0.217	0.527

TABLE 4. Content of Organic Nonsoluble Phosphorus of Nucleoprotein and Phosphatide in Various Organs of Plants Grown Under Different Nutritional Regimes (No. of counts per 100 mg dry wt.)

	Phosphorus compound	−P→+ P, plants 87-92	−P→+ P, plants 93-98	Complete nutrient mixture, plants 21-25
Roots	Organic nonsoluble	1231	1437	52.5
	Phosphatide	128	31	8.2
	Nucleoprotein	1103	1406	44.3
Leaves of first node	Organic nonsoluble	316	414	17.0
	Phosphatide	153	91	13.6
	Nucleoprotein	163	323	3.4
Leaves of second node	Organic nonsoluble	434	Removed	39.0
	Phosphatide	214		21.0
	Nucleoprotein	219		18.0
Leaves of third node	Organic nonsoluble	416		91.0
	Phosphatide	174	Removed	27.0
	Nucleoprotein	140		64.0
Leaves of fourth node	Organic nonsoluble	694		174.0
	Phosphatide	251	Removed	69.0
	Nucleoprotein	443		105.0

TABLE 5. P^{32} Content of Leaves of Buckwheat Plants Which Had Received Phosphorus through the Root System or through the Lower Leaves (No. of counts per 100 mg dry wt.)

Nutritional regime prior to application of P^{32}	Leaf node					
	lower		middle		upper	
	P^{32} added soil	P^{32} applied to lower leaves	P^{32} added soil	P^{32} applied to lower leaves	P^{32} added soil	P^{32} applied to lower leaves
N and P added during preparation of pots	539	4952	717	734	909	896
P added during preparation of pots, N added later	4602	5107	5410	767	1224	917

the experiment, all leaves except those of the first node were removed from plants 93-98. The plants were then transferred to complete nutrient mixtures containing P^{32}. At the same time plants 87-92, bearing a full complement of leaves, and plants 21-25, which had been kept on complete nutrient solutions, were also transferred. The experiment was concluded after a day and the plants killed. Results of determinations of nucleoprotein and phosphatide phosphorus in the roots and leaves of these plants are presented in Table 4.

The total amount of nucleoprotein and phosphatide phosphorus synthesized during this time is considerably

higher in plants with all their leaves than in those with only one leaf. With respect to individual amounts of the two types of organic phosphorus in experimental plants, however, the differences from the control were divergent in character. There was considerably more nucleoprotein phosphorus in the roots of experimental plants than in the roots of control plants; this pattern was especially marked in leaves of the first node. In the case of phosphatide phosphorus, however, there was a very much smaller amount in plants from which the young leaves had been removed than in control plants.

Thus, it may be supposed that removal of part of the leaves inhibits movement of metabolic products from the roots and leads to heightened synthesis of nucleoprotein phosphorus in the remaining leaves and in the roots themselves. At the same time, however, this causes a reduction in the total quantity of nucleoprotein phosphorus synthesized by these plants, as compared with plants with a full complement of leaves.

Removal of a large proportion of leaves from plants exerts a suppressive effect on synthesis of phosphatide phosphorus, since this fraction decreases considerably even in the single remaining leaf. This compels the conclusion that the chief phosphatide precursors are formed in the leaf blades. The effect of leaf removal on nucleoprotein synthesis may also be related to some metabolic product which flows into the root system. However, the explanation of an adverse effect of leaf removal may reside as well in an absence of outflow of intermediates, which is in itself a factor depressing normal rates of nucleoprotein synthesis.

Results of these experiments are not adequate to permit a conclusion as to the nature of metabolic disturbances which result from even a short-term phosphorus starvation, and which become especially pronounced with prolonged absence of phosphorus from a medium containing nitrogen.

The reason for this is the importance of the root system in the over-all process of nutrition, and therefore establishment of the specific role of roots in nitrogen-phosphate nutrition has at the same time a bearing on the whole metabolic cycle involved in plant development. Nevertheless, results obtained have made it possible to demonstrate very rapid and marked shifts in metabolism of important phosphorus compounds.
An extremely important feature of these studies is the demonstration not only of marked differences in rates of nucleoprotein and phosphatide synthesis in plants grown under various nutritional regimes, but also of marked alterations in the relative intensity of these processes in roots as compared with aerial parts. Of no less importance is the fact that the high rate of metabolism of nucleoproteins and phosphatides in leaves is dependent on a connection of these organs with root systems which had previously been deprived of phosphorus. It is, of course, impossible to give a thoroughgoing explanation of this without specifically designed experiments.

The direct dependence of vigorous synthesis of nucleoproteins and phosphatides, even in old leaves which had been deprived of phosphorus, on a rapid flow of organophosphorus compounds formed by the roots during their exposure to complete nutrient solutions is, however, most apparent.

Under ordinary conditions of nutrition, plant sap does not contain organically bound phosphorus in any substantial amount. After phosphate starvation a significant amount of organic phosphorus is always found in the sap. This change in sap composition is due to a disturbance of nitrogen nutrition in the roots [7].

The nitrogen metabolism involved in the cycling of various compounds between the aerial organs and the roots may be disturbed not only by an absence of phosphorus in the nutrient medium, but also by abrupt changes in nitrogen nutrition. One such change is a change in the P/N ratio of the nutrient medium. Subsequent incorporation of phosphorus into the medium leads to very great differences in the utilization of absorbed phosphorus. This idea was examined in a number of experiments with soil cultures.

In Table 5 are presented data from an experiment in 1949 on the P^{32} content of various leaves of buckwheat plants to which P^{32} had been applied at the height of flowering; these plants had previously been subjected to various nitrogen-phosphorus regimes. In the first group, nitrogen and phosphorus were added to the soil with which the pots were filled. In the second group, only phosphorus was added to the soil. Nitrogen salts were added after appearance of the shoots. At the time of application of radioactive phosphorus, plants of the second group were considerably better developed; they had a large number of flowers and seeds and a high fresh weight.

Radioactive phosphorus was supplied to the two groups of plants either through tubes into the soil or by application of a given volume of solution to the lower leaves.

Two days after application, P^{32} in the various leaves was determined. Results of these determinations show that rates of phosphorus absorption and distribution are substantially different in the two nutritional groups. In plants which had been supplied with nitrogen prior to sowing, a larger quantity of the absorbed phosphorus was found in the young terminal leaves. In the other plants, a considerably greater amount of the newly absorbed phosphorus was retained by the oldest leaves. When phosphorus solutions were applied to the leaves, distribution patterns were altered in comparison with plants which had absorbed phosphorus through the roots; these patterns were independent of previous nutrition. Factors determining the flow of phosphorus from leaves are substantially different from those involved in movement from the roots. Distribution of phosphorus absorbed through the leaves in relation to plant condition and to environmental factors has not yet been thoroughly studied [8, 9].

Data on phosphorus absorption, dry weight increment and seed yield for the seed ripening period were obtained. These determinations were made on parallel plants which had been supplied with nonradioactive phosphorus. Differences in phosphorus absorption rates which emerged the first two days were fully evident at the end of the experiment. The phosphorus content was very high in all the vegetative organs of plants which had been supplied with phosphorus subsequent to a post-sowing nitrogen application (P→N→P). However, phosphorus utilization by these plants was very poor in comparison with that of plants in which nitrogen was supplied prior to sowing (PN→P). This is demonstrated not only by the high percentage of phosphorus in leaves and stems, but also by the very marked lag in dry weight increment. Development was suppressed to a still greater degree. Moreover, the total quantity of phosphorus absorbed by plants of this group (P→N→P) after phosphate application was only 51.9 mg as compared with 133.26 mg absorbed by plants of the first group (PN→P) during the same period. Finally, in the month after phosphorus application 237 seeds were formed, which comprises an even greater proportion of the total yield.

The difference in final seed yield between these two groups was not especially significant. Plants of group PN→P yielded 11.65 g, and plants of group P→N→P yielded 10.43 g. These figures show especially vividly the suppressive effect of phosphorus nutrition on plants of group P→N, since at the time of phosphorus application they were markedly more advanced in the development of reproductive organs then were plants which had been supplied with both phosphorus and nitrogen prior to sowing (group PN). In this connection control plants of both groups yield information which enables one to come to a conclusion. These were plants which had continued to develop to maturity without application of phosphorus. Plants of group PN yielded only 7.88 g of seed at the end of the growing period, i. e., 3.79 g less than when there was a supplementary application of phosphorus during the fruiting period. Plants of group P→N yielded 15.79 g of seed, i. e., 5.36 g more than plants treated in the same way but with an additional application of P during fruiting.

Thus, results of this experiment have confirmed the supposition based on studies of the aftereffect of phosphate starvation that it is possible to grow plants with widely varying capacities to utilize phosphorus applied at a given time during the development period. The nutritional regime employed during the period prior to phosphorus application is a factor determining phosphorus utilization. Data obtained in studies of buckwheat permit one to conclude that the reason for such varying effects of phosphorus application resides in the character of the nitrogen metabolism, which is regulated by the nitrogen-phosphorus regime employed. Data presented above on protein and non-protein nitrogen levels in the roots of plants subjected to various nutritional regimes also indicate that specific conditions for the absorptive activity of these organs are created.

SUMMARY

1) The rate of phosphorus absorption and distribution between roots and leaves is dependent on the interchange of materials between these organs. The most basic factor is that involving nitrogen metabolism, which undergoes changes during ontogenesis and is dependent to a still greater extent on external nutritional conditions. An increase in the organic soluble nitrogen fraction of the root system ensures a marked increase in its capacity to release absorbed phosphorus to the aerial organs.

2) A factor exerting a most profound effect on the nitrogen metabolism of a plant is that of phosphorus starvation. With a change in nitrogen metabolism of the root system, there ensues an abrupt change in the rate of synthesis of the protoplasmic phosphorus compounds from phosphorus absorbed from the nutrient medium; even the metabolism of old leaves undergoes a rejuvenation in this respect.

3) In leaves of plants which had developed on a complete nutrient solution continuously, synthesis of nucleoproteins and phosphatides, as judged by the P^{32} content of these fractions, proceeds at the same rate whether phosphorus is absorbed through the roots or the leaves. Synthesis of these compounds in leaves of plants which had been deprived of phosphorus is 15-20 times higher when phosphorus is absorbed through the roots than when it is absorbed by excised leaves.

4) Elucidation of the role of roots and leaves in nucleoprotein and phosphatide synthesis is a complex problem which requires complicated experiments. Our results show that leaf blades are the major site of phosphatide synthesis, although these compounds may be synthesized in isolated root systems as well. An increase in the total quantity of nucleoprotein is to a significant degree a result of synthetic activity in the roots. Likewise, removal of a portion of the leaves results in a reduction in the plant's total nucleoprotein.

5) The pattern of absorption and utilization of phosphorus applied during growth or even at the height of the flowering period is strongly dependent on nutritional conditions prevailing during the preceding period of development.

6) In plants whose roots have lost their buffer capacity with respect to phosphorus because of an unbalanced nitrogen nutrition, leaves fulfill this function in relation to the reproductive organs.

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THE EFFECT OF MINERAL FERTILIZERS ON PHYSIOLOGICAL-BIOCHEMICAL PROCESSES IN CORN

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Mineral fertilizers have been widely employed to promote yield increase and improvement in quality of corn. However, the relationship of mineral nutrition to plant metabolism is still obscure in many respects. Frequently the effect of a given factor (mineral fertilizers) on physiological processes and yield in corn varies with conditions employed [14, 16, 18, 20]. The problem of increasing the nitrogen content of corn is an especially pressing one. In view of this we performed an investigation under field conditions of the effect of sodium phosphate and ammonium nitrate on physiological-biochemical processes in corn.

METHODS

The experiment was carried out in 1957 in the Botanical Garden of the Voronezh State University with VIR-42 corn. 5 g of superphosphate and 1.5 g of ammonium nitrate were placed in each drill hole. The fertilizer was then covered with a layer of soil 1 cm thick, after which three grains were placed in the hole and covered with soil. The soil was a saturated chernozem. Seeds were sown by hand in the square-group arrangement. The experimental plot was divided into 12 parts of 27 m^2 each. There were 80 group plantings on one division. Free and bound water contents were determined by the refractometric method; chlorophyll was determined according to the method of Godnev; respiration rate was measured with barium hydroxide in Conway flasks; photosynthesis was measured by the method of Borodulina and Kolobaeva [1]; water-retaining and water-absorbing capacity were determined by a method previously employed [2]. Catalase activity was measured according to Bach and Oparin, and peroxidase and polyphenol oxidase activity according to Mikhlin and Bronovitska; ascorbic acid content was determined by the method of Til'mans; sugar, by the iodometric method; starch, by acid hydrolysis; cellulose, according to Ermakov's modification; total nitrogen, according to Kjeldahl; protein nitrogen, with a preliminary precipitation according to Barnshtein; phosphorus, by wet ashing followed by measurement in an FÉK-M colorimeter fitted with a red filter. Samples consisted of leaves of the third node from the top taken from 5-10 plants. Samples were collected at 8-9 a. m.

RESULTS

1. The Effect of mineral fertilizers on physiological properties of corn

Mineral fertilizers exerted a marked influence on corn growth, with phosphorus being more effective on saturated chernozem soil than nitrogen (Table 1).

They also have a substantial influence on water relations. As the graph shows, nitrogen fertilizers increase the hydration of leaf tissues markedly. At the end of the growing period there was more water in the leaves of plants treated with nitrogen than in those of the control. This indicates an inhibition of the aging process in the organism [3, 4]. Phosphorus fertilizers, on the other hand, induce a reduction of leaf tissue hydration and hasten plant maturation. The marked difference in hydration of leaves of various treatment groups on the first observation date is due to the fact that there was a water deficiency in June (there were 34 mm of precipitation as compared with a yearly average of 65.4 mm).

Tissue hydration of leaves of various nodes at various times during the growing period is shown in Table 2. At the beginning of the growing period leaf water content increases from the lower nodes upward. Later, there is an increase only up to a certain node, after which there is a decrease; finally, beginning in July and continuing to the end of the growing period, hydration decreases from the lower nodes upward. Similar data have been obtained by Gushchin [5], Novogrudskii [6], and by the authors using other plants [7]. Superphosphate consistently reduced hydration at all nodes, while ammonium nitrate increased it. Although simultaneous application of nitrogen and phosphate fertilizers brought about a decrease in leaf hydration in comparison with the control, still the decrease was not as great as with phosphate fertilizer alone.

Of interest is the dependence of free and bound water contents in leaf tissues on treatment. Measurement of free and bound water showed (Table 3) that the previously observed decrease in total water content which occurs during growth is the result of a decrease in both fractions. Mineral fertilizers have differing effects on water distribution between the two fractions.

While ammonium nitrate had almost no effect on free water content and brought about a moderate increase

TABLE 1. The Effect of Mineral Fertilizers on Corn Growth (average data from 20 plants)

Date of observation and growth stage	Treatment	Height, cm	Leaf length, cm	Leaf width, mm	Av no. of leaves
6/10—leaf formation (three-four leaves)	O	15.2	13.8	15.0	3.1
	N	16.3	13.9	15.0	3.3
	P	16.2	13.7	15.1	3.4
	NP	17.3	13.9	16.2	3.6
6/20—the same (five-six leaves)	O	26.3	21.2	14.7	5.1
	N	28.1	21.6	20.4	5.4
	P	30.0	23.0	21.9	5.6
	NP	36.1	23.1	22.0	5.9
7/1—the same (seven-eight leaves)	O	49.9	39.7	45.0	7.2
	N	50.5	40.3	49.0	7.5
	P	54.7	41.0	58.3	7.6
	NP	59.5	42.1	63.8	7.7
8/10—tassel emergence	O	163.0	49.0	102.0	13.0
	N	164.1	50.1	106.0	14.0
	P	167.2	49.9	108.0	14.3
	NP	173.0	56.1	113.0	14.5

TABLE 2. Hydration of Corn Leaves According to Experimental Treatment (values in % of wet weight; leaves counted upwards)

Treatment	Leaf No.	20.VI	Leaf No.	29.VI	Leaf No.	10.VII	Leaf No.	22.VII	Leaf No.	15.VIII	Leaf No.	18.IX
O	2	82.6	2	80.8	6	80.1	6	79.2	6	75.7	6	71.2
	3	83.1	3	80.1	7	79.4	8	78.9	8	73.4	8	70.3
	4	83.9	4	81.8	8	79.2	10	78.0	10	72.2	10	69.6
	5	84.5	5	82.5	9	78.6	12	77.6	12	71.5	12	68.3
N	2	83.0	2	82.7	6	80.6	6	80.2	6	76.7	6	72.5
	3	83.5	3	82.3	7	80.4	8	79.6	8	74.4	8	71.1
	4	84.9	4	82.6	8	79.8	10	78.8	10	73.9	10	70.4
	5	85.4	5	81.3	9	79.2	12	78.2	12	72.8	12	69.7
	—		6	80.7	—		—		—		—	
P	2	80.9	2	81.4	6	80.0	6	78.7	6	74.6	6	70.3
	3	81.0	3	82.5	7	79.3	8	78.2	8	73.7	8	69.7
	4	81.2	4	81.2	8	78.6	10	77.5	10	71.9	10	68.4
	5	81.9	5	80.9	9	78.4	12	77.3	12	71.2	12	67.5
	—		6	80.2	—		—		—		—	
NP	2	80.1	2	80.5	6	80.2	6	79.4	6	74.2	6	71.6
	3	82.7	3	81.8	7	79.8	8	78.3	8	73.8	8	70.7
	4	82.6	4	82.5	8	78.7	10	77.7	10	72.9	10	69.8
	5	82.9	5	82.4	9	78.5	12	77.5	12	71.8	12	68.7
	—		6	80.0	—		—		—		—	

in bound water content, superphosphate caused a perceptible reduction in free water and an increase in bound water. Similar results were previously obtained with wheat by other workers [8]. It is interesting that when nitrogen fertilizer is applied together with phosphate fertilizer, it strongly depresses the dehydrating effect of the latter on protoplasmic biocolloids.

The water-retaining and water-absorbing capacities of leaves are markedly altered (Table 4). Fertilizers have an enhancing effect on both processes. Nitrogen fertilizers always have a smaller effect than phosphate fertilizers. With plant aging, there is a sharp reduction of water-retaining capacity and an increase in water-absorbing capacity of leaves. We have previously observed this phenomenon with other plants [2].

The water relations of corn leaves are intimately related to other physiological processes—photosynthesis, respiration, etc. Measurement of the rate of photosynthesis showed that this process is also subject to alteration. Mineral fertilizers stimulated it throughout the entire growing period. It should be pointed out that photosynthetic rate constantly rises up till the flowering peri-

od, and then falls regardless of experimental treatment.

Leaf chlorophyll content (in % of dry weight) varied with experimental treatment as follows:

Treatment	8/15	9/18
O	1.023	0.984
N	1.275	1.242
P	1.186	1.040
NP	1.208	1.118

Mineral fertilizers also bring about an increase in the sugar content of leaves (Table 5). Sucrose is accumulated to a greater extent than is glucose. In general, an improvement in growing conditions is manifested primarily in sucrose biosynthesis, which indicates its importance in plant metabolism [9, 4, 10].

An increase in sugars resulting from application of mineral fertilizers is accompanied by a certain increase in respiration rate and in ascorbic acid content.

The respiration rate of corn leaves (in mg CO_2 per dm^2 per hr) measured on July 10, 1957, was as follows:

Treatment	CO_2 released	Treatment	CO_2 released
O	1.46	P	1.53
N	1.49	NP	1.55

Ascorbic acid content of corn leaves (in mg per 100 g wet wt.) at various periods of plant development and with various experimental treatments was as follows:

Treatment	6/22	7/10	7/26	8/10
O	48.1	48.1	50.3	49.7
N	48.5	50.9	52.6	51.6
P	50.5	51.1	55.3	54.5
NP	51.1	51.2	61.9	56.3

Ascorbic acid content changed during the growing period in a manner characteristic of other plants [11].

Catalase, peroxidase and polyphenol oxidase play an important role in plant metabolism. Quantitative changes in these enzymes are closely related to the most important aspects of plant activity. As our studies show, mineral fertilizers have a stimulating effect on these enzymes.

Catalase activity of corn leaves (in ml 0.1 N $KMnO_4$ per hr per g wet weight) at various stages of development and with various experimental treatments was as follows:

Treatment	6/27	7/12	7/27	8/12
O	55.9	63.7	84.1	74.4
N	56.7	64.8	85.5	76.2
P	57.5	66.1	86.7	76.2
NP	59.7	67.2	88.2	76.2

It is characteristic that the dynamics of change in catalase activity during the growing period are reminiscent of those for other physiological processes such as respiration, photosynthesis, ascorbic acid accumulation, etc., with which processes it is apparently closely involved [2, 4, 10, 12].

TABLE 3. Free and Bound Water Contents of Corn Leaves According to Experimental Treatment (in %)

Treatment	6/29, 4th leaf		8/18, 10th leaf	
	free	bound	free	bound
O	69.60	12.20	58.65	10.65
N	69.55	16.05	57.85	12.55
P	64.25	18.55	53.40	15.20
NP	67.85	14.75	56.70	13.10

TABLE 4. Water-Retaining and Water-Absorbing Capacity of Corn Leaves According to Experimental Treatment (in % of wet weight)

Treatment	6/18		6/15	
	water-retaining	water-absorbing	water-retaining	water-absorbing
O	60.4	6.2	34.8	16.1
N	62.1	6.5	38.6	17.4
P	63.9	9.6	46.7	18.7
NP	64.6	10.7	49.2	19.5

It should be noted that, in contrast to polyphenol oxidase, the activity of peroxidase increases sharply to about the middle of July when mineral fertilizers have been applied (Table 6). Subsequently its activity fluctuates slightly. These changes in individual enzymes indicate, first, that they have varying sensitivities to mineral fertilizers and, second, that there may be reciprocal changes in types of aerobic respiration [13].

2. The Effect of Mineral Fertilizers on Chemical Composition and Yield of Corn

The chemical composition of corn is extremely variable and depends on many factors—variety, climatic conditions, fertilizers, etc. It is possible to influence not only the quantity of yield but also its quality by application of fertilizers. Our studies of the chemical composition of leaves and grains of corn have shown that phosphate and nitrogen fertilizers have a considerable effect on the formation of many of its important compounds.

Nitrogen and phosphorus content (N and P_2O_5 in mg per g air dry weight) of the 11th leaf from the bottom, measured in a sample collected September 3, varies with experimental treatment as follows:

Treatment	Total nitrogen	Phosphorus
O	20.76	2.04
N	22.12	2.18
P	22.05	2.45
NP	24.20	2.14

The content of total nitrogen and of phosphorus in the leaves at the time of harvest for silage was considerably higher where nitrogen and phosphate fertilizers had been applied.

TABLE 5. Sugar Content of Corn Leaves According to Experimental Treatment (in % of air dry weight)

Treatment	8/10			8/25		
	Glucose	Sucrose	Total	Glucose	Sucrose	Total
O	5.30	1.29	6.59	5.04	1.09	6.13
N	5.33	1.36	6.69	5.12		
P	5.45	1.50	6.95	5.36		
NP	5.72	1.62	7.34	5.42		

TABLE 6. Peroxidase and Polyphenol Oxidase Activity in Corn Leaves (in ml 0.01 N $KMnO_4$ per g wet weight)

Treatment	7/2		7/3		7/27		8/12	
	Polyphenol oxidase	Peroxidase	Polyphenol oxidase	Peroxidase	Polyphenol oxidase	Peroxidase	Polyphenol oxidase	Peroxidase
O	7.75	9.25	9.63	10.66	11.11	11.63	10.42	10.93
N	8.21	9.32	11.22	16.72	12.14	17.11	10.45	16.81
P	9.11	9.42	11.44	17.63	12.31	18.13	11.31	17.77
NP	9.52	10.41	11.92	22.12	12.74	23.31	11.32	20.22

Grain quality showed a satisfactory improvement with application of these fertilizers. As is shown in Table 7, both ammonium nitrate and superphosphate stimulated the accumulation of sugars, starch and nitrogenous materials in the grain and reduced to some extent the content of cellulose.

When nitrogen fertilizer was used, there was a higher content of nitrogenous materials in the grain than when phosphate fertilizer was used. The quantity of protein nitrogen was markedly increased, especially with combined application of fertilizers. There are contradictory data in the literature on the effect of mineral fertilizers on protein biosynthesis. Thus, Genter and co-workers [14] observed an increase in protein content of the grain with nitrogen fertilizers and no effect with phosphate and potassium fertilizers. Kolosha [15] also reported similar results; in his experiment, however, simultaneous application of nitrogen and phosphate fertilizers even caused a reduction of protein in the grain. Other workers report that there is no variation in chemical composition of the corn grain [16-18].

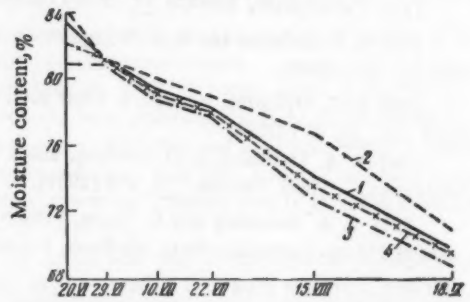

Figure. The effect of mineral fertilizers on hydration of corn leaf tissues (third node from the top). 1—control; 2—with nitrogen; 3—with phosphorus; 4—nitrogen plus phosphorus.

In our experiments, application of nitrogen and phosphate fertilizers promoted an increase in soluble nitrogen of the grain to a greater degree. We encountered in the literature, however, indications that enhanced phosphorus nutrition is associated with an increase in protein synthesis and a decrease in the nonprotein nitrogen fraction [18-20]. As our data show, we observed a somewhat different pattern. A high ratio of nonprotein nitrogen to protein nitrogen under optimal external conditions, as has been shown to occur by certain workers [21], may serve as a diagnostic criterion of an ample supply on mineral elements.

We observed no marked changes in fat content of the grain. We find similar results to have been obtained by other workers [14]. Total ash content shows little alteration with application of fertilizers. At the same time, there is more phosphorus in the grain of plants given experimental treatments, which is indicative of an improvement in the grain's food quality.

Mineral fertilizers exerted an influence on grain yield as well as on grain quality (Table 8).

With combined application of nitrogen and phosphorus fertilizers, the total amount of grain nitrogen produced per hectare is doubled and the protein is increased to a value one and a half times the control.

SUMMARY

1) During development of corn plants, there is an increase in the rates of physiological processes occurring in the leaves from the beginning of the growing period to the flowering stage; subsequently these rates decline to the end of the growing period.

2) During the growing period, leaf hydration constantly decreases. Nitrogen fertilizer impedes this decrease, while phosphate fertilizer hastens it. Until the 4-5 leaf stage is reached, leaf hydration increases as

TABLE 7. Chemical Composition of the Corn Grain (in % of absolute dry weight, February, 1958)

Treatment	Sugars			Starch	Cellulose	Total carbohydrates	Nitrogen				Fat	Ash	Phosphorus
	glucose	sucrose	total sugars				total	protein	soluble	ratio of soluble nitrogen to protein nitrogen			
O	3.51	1.23	4.74	52.11	2.72	59.57	2.18	1.61	0.57	0.35	3.84	0.27	1.77
N	3.66	1.35	5.01	52.16	2.53	59.70	2.64	1.69	0.95	0.56	3.83	2.91	1.86
P	3.91	1.49	5.37	53.12	2.44	60.93	2.43	1.65	0.78	0.47	3.82	0.32	1.83
NP	3.98	1.51	5.49	54.51	2.23	62.23	2.82	1.78	1.04	0.59	3.84	0.33	1.87

TABLE 8. Grain Yield and Gross Yield of Nitrogenous Materials According to Experimental Treatment

Treatment	Grain wt.		Total nitrogen		Protein	
	cwt/hectare	%	kg/hectare	%	kg/hectare	%
O	36.3	100	67.7	100	350	100
N	38.7	106	102.2	146	392	112
P	41.4	114	106.0	156	409	116
NP	50.1	138	141.3	208	535	152

leaf height increases; subsequently it diminishes with the appearance of new leaves.

3) Mineral fertilizers promote an increase in bound water content and in water-retaining and water-absorbing capacity of leaves.

4) Application of nitrogen and phosphate fertilizers, especially in combination, leads to an increase of total and protein nitrogen, sugar, starch and phosphorus in the corn grain, and to a decrease in cellulose. Fat content does not undergo any marked changes. Total nitrogen production per unit area is more than doubled.

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THE SPECIFIC SIGNIFICANCE OF THE ROOTS FOR GROWTH OF THE AERIAL PORTION OF THE PLANT

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The specific significance of roots for shoot growth was first noted by Went in etiolated pea seedlings [1], and was later shown in rye seedlings by de Ropp [2], in Rudbeckia by Chailakhyan [3], in lemon by Tumanov and Vinokur [4], and in potato by Gupalo and Litvak [5].

In addition, it was established by the studies of Sabinin and co-workers begun in 1927 [6], of Shmuk, Smrinov and Il'in [7], of Dawson [8], and of Mothes [9] and others that the root system is not only an organ for absorption of water and mineral nutrients from the soil, but also specific site of synthesis of a number of organic compounds.

What substances, synthesized in the roots, are responsible for their specific influence on the growth of aerial organs?

That amino acids are synthesized in roots was firmly established by a number of studies [6, 10]. Kursanov [11] observed a relatively high amino acid content in the aerial roots of Ficus, and Dadykin and Igumnova [12] found the same thing in the prop roots of corn. This led to the view that amino acids, or perhaps some of them, are the compounds responsible for the decisive effect of roots on the growth of aerial organs, although Sabinin [13] was more inclined to the supposition that nucleic acids and their components are the substances in question.

Our goal was to investigate this problem using germinating potato tubers, in which the roots had been shown to play a determining role in shoot growth. At the same time, it was of considerable interest to clarify the physiological-biochemical basis of shoot growth in potato during etiolation, at which time the roots do not participate, as shown in the study of Miroshnichenko [14].

1. Experiment With Potato Tuber Halves

The method used was one developed earlier by us [5] with certain improvements.

On May 29, 1956, 50 potato tubers of the variety Seyanets-36 were placed in the light in the laboratory for preliminary sprouting and selection. After a few days, the upper and lower buds were removed and only the buds in two symmetrically placed eyes in the central part of the tuber were left.

The tubers were kept in the light for 31 days more. All the newly sprouting buds were removed, and only the sprouts from the two centrally located eyes were retained.

On June 29 the tubers were disinfected with 1% formalin and then cut into halves of equal weight; weights of the two halves were equalized by removal of a thin tissue layer from the cut surface where necessary. Half of each tuber was placed on glass rods in a Petri dish containing water so that the sprout was high enough above water to prevent root formation but was able to obtain water through the tuber. The other half was covered with sand which had been thoroughly roasted and washed with hydrochloric acid; conditions were thus provided to promote formation of roots which could obtain no mineral nutrients from the external environment, only water.

During the experiment the temperature was maintained at 22-24°. The tubers were watered daily; water in the Petri dishes was changed every two or three days.

Fig. 1. Growth of potato sprouts from halves of a single tuber without roots (in Petri dish) and with roots (in evaporating dish).

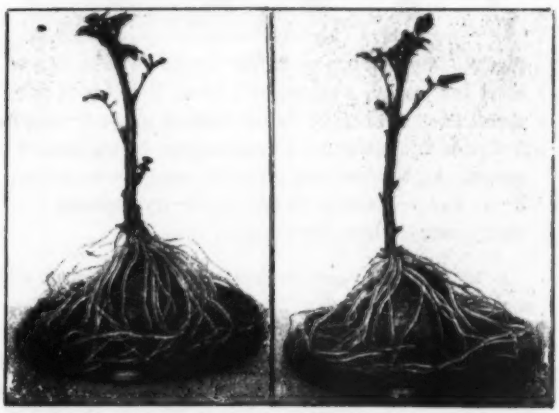

Fig. 2. Root system developed in moist sand in 14 days.

and the suberized layer of cells was removed from the cut surface periodically.

On July 14 the plants were photographed (Fig. 1), and material was collected for analysis of protein and nonprotein nitrogen, and also for determination of amino nitrogen and chromatography of free amino acids.

As Fig. 1 shows, after 14 days sprouts from those tuber halves which were in moist sand, i.e., which had the opportunity to form roots, had grown to a length of 100-200 mm. Shoots of control halves, however, showed almost no change and had a total length of 20-30 mm in spite of the fact that there was an adequate supply of water and that nutrient materials in the tuber were available to them. The density of the root system formed during this period in sand is shown in Fig. 2.

Samples of shoots, roots and tuber tissue under the eye were analyzed separately. Shoots were split lengthwise and one half was used for determination of protein and nonprotein nitrogen, while the other was used for determination of free amino acids. Root and tuber tissue samples were divided in two parts in the same manner. Each sample comprised material from five plants.

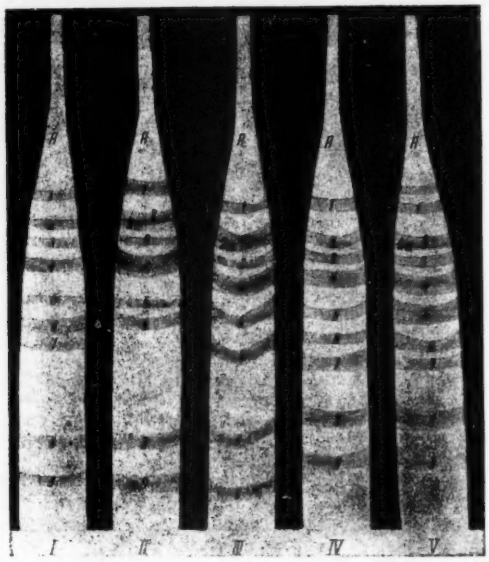

Fig. 3. Chromatograms of free amino acids in shoots, roots and tubers. I—shoot with roots; II—roots of this shoot; III—its tuber; IV—shoot without roots; V—its tuber. 1—lysine and histidine; 2—asparagine; 3—arginine; 4—aspartic acid, serine and glutamic acid; 5—threonine; 6—α- and β-alanine; 7—proline; 8—tryptophane, methionine, valine; 9—leucines.

Samples to be used in determination of protein and nonprotein nitrogen were fixed with hot steam and then dried at 60-70°. Those to be used for determination of free amino acids were ground in 80% ethyl alcohol, and the brei was made up to a final volume corresponding to 1 part tissue wet weight in grams to 20 parts alcohol in milliliters.

The content of protein and nonprotein nitrogen was determined by the Kjeldahl method with proteins being precipitated according to Barnshtein as described in Ermakov [15]. First, amino nitrogen of the alcoholic extracts was determined by the ninhydrin method, according to Pochink and Bershtein [16], and then free amino acids were separated by paper partition chromatography and identified with the help of ninhydrin.

For greater reliability the experiment and the amino acid chromatography were repeated three times during 1956 and 1957. Substantially similar results were obtained all three times; by our request, in one replicate experiment determination of amino nitrogen and the two-dimensional chromatography were carried out by B. I. Bershtein in the biochemistry laboratory of the Institute of Plant Physiology, MSKh, USSR (director of the laboratory, A. S. Okanenko).

Data from the biochemical studies are summarized in Table 1 and in Figs. 3 and 4.

Table 1 shows that the percentage of total and protein nitrogen is higher in the sprouts and tuber on which no roots were formed (on water) than in the sprouts, roots and tubers on which roots were formed (in sand). This is evidently due to the fact that where roots were formed there was heightened growth activity, and therefore an increase in dry matter content as a result of synthesis of cellulose, which became relatively more abundant.

Chromatograms of free amino acids (Figs. 3 and 4) showed that the amino acid composition is the same in shoots and tubers without roots as in those with roots. It is true that on the two-dimensional chromatogram β-alanine was found in the shoot with roots and not in the one without roots, but in the roots themselves this amino acid was absent, possibly because it occurred in relatively small quantities. This is evidently the explanation of the fact that on the two-dimensional chromatogram threonine and serine were absent in the shoot with roots and present in traces in the shoot without roots.

In all replicates it is very striking that there was a comparatively large quantity of proline in shoots and tubers without roots, while in shoots with roots this compound occurred in very small amount, and in the roots it was not found at all.

Thus, we were unable to find either a quantitative or a qualitative deficiency of amino acids in shoots whose growth was inhibited by the absence of roots. It may be supposed that when sprouting without root formation occurs in potato, a sufficient quantity and diversity of amino acids is formed by the action of proteases. All the same, however, some factor is lacking, and therefore normal growth does not occur until roots are formed, even though they obtain no nutrients from the external environment other than from the tuber. It is possible that nucleic acids or their components are the factors in question, as Sabinin suggested [13]. This problem should be resolved by further studies.

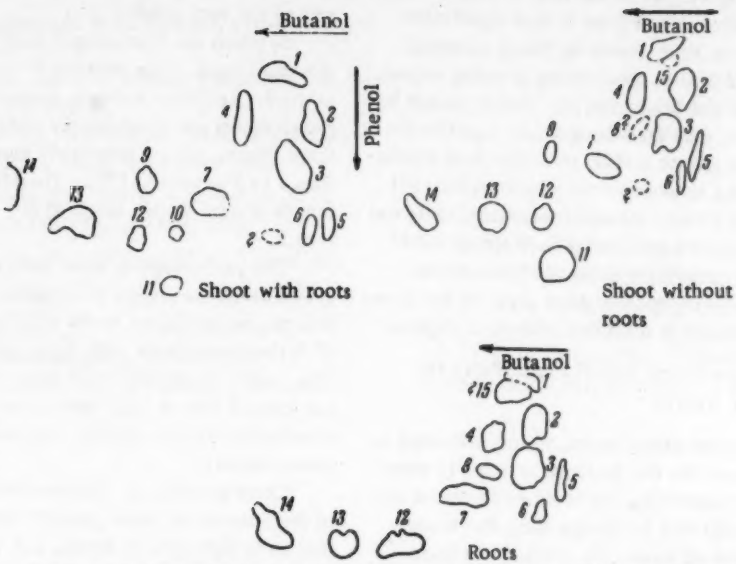

Fig. 4. Chromatograms of free amino acids in shoots with and without roots, and also in roots (made by B. I. Bershtein). 1—Aspartic acid; 2—asparagine; 3—glutamine; 4—glutamic acid; 5—histidine; 6—arginine; 7—α-alanine; 8—threonine (?); 9—tyrosine; 10—β-alanine; 11—proline; 12—γ-aminobutyric acid; 13—valine, tryptophane, methionine; 14—leucines; 15—serine.

TABLE 1. Amino Nitrogen and Protein Content of Shoots, Roots and Tubers

Item measured	In sand			On water	
	shoots	roots	tuber	shoots	tuber
Nitrogen, percentage of dry matter:					
total	3.15	2.74	1.30	3.92	1.51
protein	2.54	2.28	1.17	3.49	1.19
nonprotein	0.61	0.46	0.43	0.43	0.32
Protein, percentage of dry matter.	15.87	14.25	7.31	21.81	7.43
μg amino nitrogen per g dry matter:					
a) in expt. of 1956	52	41	110	138	138
b) in expt. of 1957	55	23	100	115	90

TABLE 2. Characteristics of Light-Grown and Dark-Grown Shoots of Potato

Item measured	Light-grown shoots	Etiolated shoots
Av shoot length, cm	2	12
Wet wt. of 10 shoots, g	10.64	20.0
Dry wt. percentage	24.95	14.7
Dry wt. of 10 shoots, g	2.65	2.94
Nitrogen, percentage of dry wt..		
total	4.12	3.26
protein	3.08	2.01
nonprotein	1.04	1.25
Wt. of protein in dry mass of 10 shoots, g	0.510	0.360
Dimensions of parenchyma cells, μ:		
a) width	120	60
b) length	170	430

We have no misgiving as to the data of Kursanov[11] and Dadykin [12], since as a matter of fact significant amounts of free amino acids should be found in young growing rootlets, and also in other young growing organs, as shown by Pochink and Bershtein [16]. But it cannot be concluded from these data that the specific significance of the roots for shoot growth resides in amino acid synthesis, since it was shown in another study by Dadykin [17] that in the leaves of 40-day old wheat seedlings there was a greater diversity and a higher content of amino acids than in the roots. The relative amount of free amino acids is determined by the growth stage (age) of the given organ and by its correlative relations with other organs.

2. Study of Growth of Etiolated Potato Shoots Without Roots

In the course of our experiments, which indicated an obligatory requirement for the presence of roots in shoot growth during potato sprouting, we often encountered objections on the grounds that in storage long shoots are formed in the absence of roots. We studied etiolated shoots formed in storage cellars and established that they are always formed with the participation of roots which here arise as a result of conditions of high relative humidity. The questions arise: 1) How long will etiolated shoots be if the formation of roots is prevented, and 2) what is the anatomical-physiological basis of etiolation growth? Why can this type of growth occur in the absence of roots? In order to answer these questions, we performed the following experiment.

On May 29, 1956, 30 pairs of identical potato tubers, variety Seyanets-36, were selected. On the same day, 30 of these were placed in dark chambers (boxes); small cups filled with calcium chloride were placed in the boxes to reduce the humidity and thereby prevent root growth. The other 30 tubers were placed in the light in the same room, i.e., at the same temperature. On July 4, the length of shoots from the apexes of tubers of both groups was measured, and they were examined microscopically*. On July 13 the water content of the shoots was determined (by drying at 90-100°) and the entire mass of shoots was dried for subsequent analysis for six hours at 50-60° (after fixation with hot steam) and then for two hours at 70-80°. This material was stored in flasks with ground glass stoppers.

In September the material was analyzed for total nitrogen and for protein and nonprotein nitrogen (Kjeldahl method, with protein precipitation according to Barnshtein). The results are presented in Table 2.

Our observations showed that in this variety under the conditions employed, etiolated shoots can grow comparatively little in the absence of roots, attaining a length of about 12 cm.

Their cells are greatly elongated and have a high water content in comparison with light-grown shoots. While the wet weight of 10 dark-grown shoots is almost twice that of light-grown shoots, dry weights of the two groups are very similar.

Sections for microscopic study were made 1 cm from the shoot apex. Five sections from each group were examined. As Table 2 shows, parenchyma cells of light-grown shoots are considerably wider than those of etiolated shoots, but are relatively short, as was also established by Ehrendorfer [18]. Therefore, the increase in length of dark-grown shoots is due to an increase in cell length.

The percentage of total and protein nitrogen in dark-grown shoots was lower than that of light-grown shoots; this can be attributed to the relative increase in cellulose of dark-grown shoots with their greatly elongated cells. The weight of protein in 10 dried dark-grown shoots does not exceed that of light-grown shoots; indeed, it is even smaller (0.360 g in dark-grown and 0.510 g in light-grown shoots).

Consequently, in etiolated shoots which are growing in the absence of roots, protein formation is just as limited as in light-grown shoots, and even more so. The fact of intensive growth during etiolation, therefore, does not vitiate the hypothesis of a specific significance of roots for normal growth of aerial organs with rapid synthesis of protoplasmic proteins.

SUMMARY

1) Using a method previously developed by the author, it was shown that in light-grown potato shoots, whose growth is suppressed by the absence of roots, the amino acid complement is the same as in normal shoots growing in the presence of roots; moreover, these amino acids are available in sufficient quantity.

2) Etiolated shoots reach a considerable length only when roots are formed, even though they are formed in air.

3) The amount of protein and of nonprotein nitrogen in etiolated shoots formed without roots does not exceed that in light-grown shoots whose growth is inhibited. Therefore, the formation of proteins in etiolated shoots without roots is no more vigorous than in light-grown shoots.

4) It is necessary to continue to study the substances which are responsible for the specific significance of roots in growth of aerial organs.

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A STUDY OF THE CAUSES OF DIVERSITY IN GERMINATION BEHAVIOR OF COTTON SEEDS

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A knowledge of the causes of cotton seed diversity which is grounded in a comprehensive and deep understanding of the biological peculiarities of cotton is of great importance in modifying the high temperature requirements of this crop.

Seed diversity may be observed in many species of cultivated plants. Michurin has well described the physiological diversity of seeds from a single plant and even from a single fruit which occurs in fruit crops [1]. In studies on cotton [2-6], the opinion has been ventured that developmental peculiarities of the plants in combination with the complex of external and internal factors account for the diversity of seeds formed.

In studies of the effect of seed diversity on productivity of the progeny in cotton it is observed that the seeds which are most valuable commercially are formed at the lower and middle nodes in places situated near the main stem [7-10]. Mukhamedzhanov [11] devotes special attention to the fact that when cotton is planted comparatively early the first buds and flowers appear prior to the advent of high summer temperatures; this provides greater assurance that bolls at the lower nodes will remain intact than when planting is done later. It is also of importance that ripening and dehiscence of the bolls formed earlier occurs under especially favorable conditions. Lysenko [12] and Rzhevskii [13] point out the importance of favorable temperature conditions during development of bolls at the first nodes in their effect on the rapidity and completeness of maturation. Omel'chenko [14] came to the conclusion that accumulation of dry matter in the embryo is greatly retarded when the bolls are formed late. This is correlated with the degree of embryo viability, which depends on embryo age and on time of formation.

In studies of wheat seed diversity, the authors express the opinion that the biological character of spikelets in various parts of the head is determined by external conditions prevailing at the time of formation of the rudimentary head and also by interrelations of physiological processes [15-17].

In earlier studies we established a sharp diversity of cotton seeds from both crude and selected lots with respect to germination vigor, rate of sprouting, and seed qualities [9, 18, 19].

Seed diversity with respect to germination vigor and rate of sprouting is especially evident when there is a combination of unfavorable environmental conditions (low temperatures, water deficiency, etc.) at the time of sowing. We believe that seeds capable of germinating and producing shoots under unfavorable conditions are of great value, since the ability of such seeds to thrive under these conditions is probably inherited. The necessity therefore arises to study the biological and environmental factors which determine the ability of seeds to germinate at comparatively low temperatures. We investigated this matter at the Tashkent Agricultural Institute in the period 1948-1954. For the experiments the commercial varieties 108-F and S-460 were used.

It is characteristic of cotton that it enters the reproductive stage at the beginning of June and continues to form reproductive organs until the fall frosts. Formation of fruit, therefore, occurs under varying environmental conditions.

For illustration let us consider Fig. 1, which is based on data obtained in 1948. This example, despite the frequent climatic changes, is, in general, typical of any given year.

Figure 1 shows the pattern of change of weather conditions during the growing period of cotton, and demonstrates that each stage of plant development corresponds to a definite combination of environmental conditions.

The appearance of the first buds, and subsequently of flowers, occurs during the period that the average daily temperature is rising. At this time humidity, cloudiness, and precipitation are decreasing. Development of the first bolls coincides with the hottest period of the summer. Nevertheless, subsequently formed bolls ripen and dehisce at a lower temperature and an increased humidity.

Thus, changing environmental conditions promote in sequence the requisite physiological changes associated with each successive developmental period. Moreover, in the plant itself the assimilation-dissimilation process varies with each stage of development. We have clarified the role which interaction of cotton plants with environment plays in subsequent seed germination at comparatively low temperatures.

Seeds of variety 108-F were sown on two dates, April 17 and May 10. The dates of 50% flowering and 50% maturation of bolls in each group were recorded. The day of maturation was taken as that on which dehiscence occurred. Temperature conditions prevailing in

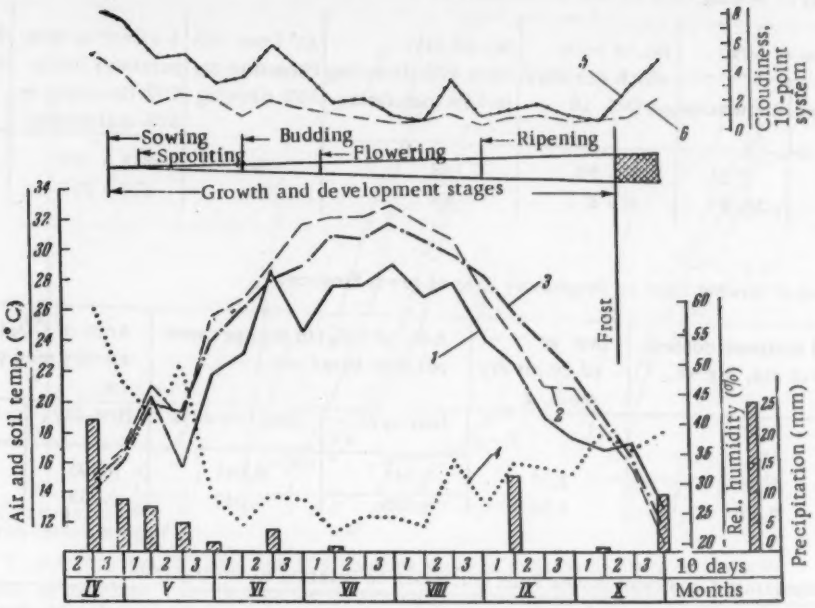

Fig. 1. Times of inception of principal growth and development stages in S-460 cotton as correlated with seasonal climatic conditions. 1—air temperature; 2—soil temperature at 10 cm; 3—soil temperature at 20 cm; 4—humidity; 5—total cloudiness; 6—low-lying cloudiness

the period from flowering to ripening were noted. On the day of the first autumn frost (October 17), on certain plants selected from each group as most typical, the number of bolls which had dehisced the day before were counted, and then the raw cotton formed prior to the frost was collected.

In subsequent experiments, the seeds obtained were kept at a temperature of 12-14° until they had germinated to their full capacity. Seeds were germinated in Petri dishes (12.5 cm in diameter) on saturated filter paper after a soaking of 24 hours. The experiment was performed in triplicate with 100 seeds in each replicate group.

Data obtained in these experiments showed that seeds from plants which began growth on the earlier data germinated to a greater extent at 12-14° than did seeds from plants of the later group (Table 1).

Part of the seeds collected were germinated at 30° in order to determine respiration rate. Determinations were made by a previously described method [19] for a day and for two days after the beginning of germination (Table 2).

Evidently the reduction in germination vigor and respiratory activity of seeds from plants sown in May depends to some degree on temperature conditions prevailing during the period of fruit formation. Our data showed that development and maturation of the first bolls on plants sown April 17 took place at quite high average daily temperatures, while development of bolls on plants sown on May 10, during the last 30 days of the flowering to maturation period occurred at considerably reduced temperatures (Table 3).

Because of changes in temperature conditions the intermediate period from flowering to boll maturation was prolonged 13 days for plants sown on the later date.

If it is assumed that in these varieties the lower temperature limit for the flowering-maturation stage is 12-13°, then even when this stage is prolonged the sum of effective temperatures for its completion differs from that where plants were sown earlier by less than 136-147°•. Boll dehiscence occurring at a lower sum of effective temperatures is evidently related to factors associated with low temperature, which manifest themselves in poor seed quality as shown by the temperature requirements for germination.

We also observed a change in the duration of the flowering—maturation period in variety S-460 (sown on April 18), which is related to the temperature factor and which depends on the time and therefore the place of boll formation on the plant. All flowers which appeared during the growing period were labeled. During the maturation period, the dates of dehiscence of the intact bolls were recorded. For each group of bolls, based on 2- to 3-day flowering intervals, the number of days from flowering to maturation was recorded, and also the

• No special determinations of the lower temperature limit were made. We assume on the basis of the work of T. D. Lysenko [12] that it (constant B) is 12-13° for our varieties. We have shown that in the event of insignificant random deviations in this constant the general pattern manifested in our studies is not disturbed.

TABLE 1. Effect of Sowing Date on Boll Formation and Seed Germination at 12-14° in Variety 108-F

Sowing date	Date of 50% flowering	Date of 50% maturation	No. of bolls which dehisced on Oct. 16	No. of days from 50% flowering to 50% maturation	Σt° from 50% flowering to 50% ripening	Σ effective temperatures from 50% flowering to 50% maturation	% of germinated seeds
4/17	7/6	8/31	15 - 20	56	1485	813 - 757	47.3
5/10	7/26	10/3	6 - 8	69	1504	677 - 610	21.3

TABLE 2. Effect of Sowing Date on Respiratory Rate of Seeds Produced

Sowing date	Seed moisture content, in % of abs. dry wt.	Av wt of 10 air dry seeds, g	Amt. of CO_2 (in mg) per seed per hour based on:		Amt. of CO_2 (in mg) per g air-dry seed per hr based on:	
			first day	first two days	first day	first two days
4/17	7.14	1.37	0.041	0.045	0.303	0.334
5/10	7.07	1.38	0.035	0.047	0.253	0.338

TABLE 3. Temperature Conditions during Fruit Formation in Relation to Sowing Date

Sowing date	Date of 50% flowering	Date of 50% maturation	Duration of period	Av daily temp. of each successive 10-day period from the time of 50% flowering						
				1	2	3	4	5	6	7
4/17	7/6	8/31	56	27.2	29.3	26.8	27.3	23.8	23.4	17.7
5/10	7/26	10/3	69	26.8	27.3	23.8	23.4	17.7	16.5	16.9

TABLE 4. Effect of Date of Boll Formation on Duration of Period from Flowering to Maturation

No. of fruits counted	Actual flowering date	Av maturation date	No. of days from flowering to boll maturation	Σt° during flowering–maturation period	Σ of effective temperatures during flowering–maturation period within assumed limits
10	7/7	9/1	56	1479	807 - 751
12	7/9	9/4	57	1498	814 - 757
26	7/11	9/7	58	1511	815 - 757
31	7/14	9/11	59	1495	787 - 728
29	7/16	9/13	59	1468	760 - 701
27	7/18	9/16	60	1450	731 - 673
27	7/20	9/20	62	1466	723 - 663
21	7/23	9/28	67	1509	706 - 641
18	7/26	10/4	70	1526	687 - 619
17	7/29	10/10	73	1521	645 - 572
8	8/1	10/17	77	1539	615 - 538

sum of average daily temperatures and effective temperatures during this period**.

A study of the duration of the period from flowering to maturation as it depends on the time and place of boll formation on the plant revealed that the later flowers appear, the longer it takes for their bolls to mature (Table 4).

In this variety, as in variety 108-F, the sum of average daily effective temperatures over the flowering-maturation period is consistently decreased for fruits formed at later times in spite of the prolongation of this period.

** The lower temperature limit for boll maturation in var. S-460 was taken as 12-13°.

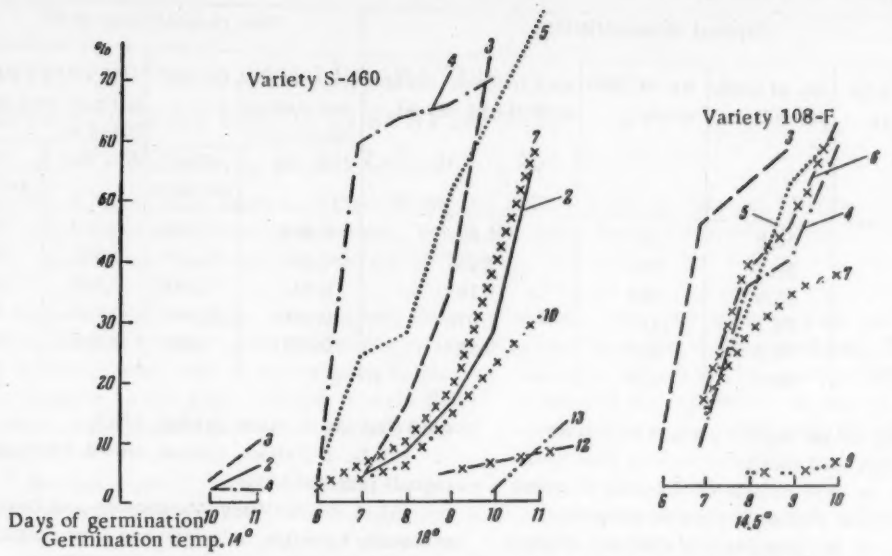

Fig. 2. Germination of S-460 and 108-F cotton seeds in relation to place of boll formation on the plant. Numbers on the curves denote positions of branches from which bolls containing the seeds were taken.

We followed the germination behavior of seeds with reference to boll development as it depends on place of formation and temperature conditions.

Seeds of variety S-460 were taken from the lowest bolls on homologous fruiting branches 2, 3, 4, 5, 7, 10, 12, 13 and pooled according to branch of origin. Seeds of variety 108-F collected from branches 3, 4, 5, 6, 7, and 9 were also germinated.

Results of the experiment showed that S-460 seeds germinated very slowly at a temperature low for this variety (Fig. 2).

At a temperature of 14°, on the 10th-11th day of germination only seeds from the 2nd, 3rd, and 4th fruiting branches had germinated to even a perceptible degree. At a higher temperature (18°), seeds from all branches germinated. Here it was possible to detect more clearly differences in germination behavior in relation to place of boll formation. Figure 2 shows that seeds from bolls of the lower and middle nodes germinate more vigorously than those from bolls of the upper nodes. Seeds from bolls formed late in the growing period do not generally germinate at low temperatures, even though the bolls dehisce before frost occurs, and at a temperature suboptimal for the given variety they germinate very weakly.

As a basis for diversity in germination behavior we present physical and physiological characteristics of 108-F seeds in Table 5.

As Table 5 shows, the place of boll formation on the bush fully accounts for the changes occurring within the fruit. For example, at the higher branching axils the average weight of raw cotton per boll is higher. In addition, the number of seeds per boll is increased, this being the chief cause of the increase in boll size. The increase in seed number is apparently due to favorable conditions. In this case, however, a paradox arises—the plant is not always able to provide many of the seeds in the boll with sufficient nutrients, and as a result they do not attain a weight as high as that of seeds from lower nodes. Especially small seeds are formed on the uppermost fruiting branches, where it is obvious even to casual observation that they are abortive.

The pattern of increase in seed number per boll and of decrease in their absolute weight with height of the plant is related to germination behavior and to respiratory activity during germination.

As seen in Table 5, in the first days after swelling seeds of the lower nodes respire more rapidly than seeds of the upper nodes. As our experiments showed, during this period seeds of the upper nodes begin to respire vigorously. Only in the following days, however, do these seeds begin to catch up with those from lower nodes in germination. At the same time there is a marked increase in their respiratory activity. Seeds from the lower nodes maintain a high level of activity throughout the entire period of early development (germination and radicle growth).

In conclusion, turning to Fig. 1, we take into consideration the fact that in striving to attain the earliest dates of bud formation, flowering and fruit maturation there is a definite limit, in time, earlier than which it is impossible for these stages to begin. They may, however, be shifted indefinitely to later dates, this being accomplished chiefly by moving up the planting time. In the former case, time interval from the inception of flowering to the first fall frost is the longest possible and embraces the days with the highest temperatures.

TABLE 5. Physical and Physiological Characteristics of Cotton Seeds in Relation to Location of Bolls on the Bush

Fruiting branch No.	Physical characteristics				Rate of respiration at 30°			
	size of bolls (wt. in g)	No. of seeds per boll	wt. of 1000 seeds, g	seed moisture content in % of abs. dry wt.	amt. of CO_2 (in mg) per seed per hr based on:		amt. of CO_2 (in mg) per g of seed per hr based on:	
					first day	first two days	first day	first two days
3	7.0	32.8	135	7.32	0.044	0.049	0.330	0.368
4	7.2	33.7	135	7.22	0.042	0.047	0.323	0.345
5	7.4	35.3	134	7.10	0.041	0.048	0.306	0.345
6	7.6	36.1	134	7.07	0.040	0.045	0.291	0.320
7	7.8	36.7	133	7.22	0.031	0.044	0.225	0.336
9	7.3	38.0	129	–	–	–	–	–

This is responsible for the highest percent of boll formation and for high seed quality.

In this study we have considered diversity of cotton seeds as related to the pattern of plant development, which is affected by the progression of climatic changes throughout the growing season, and also as related to the location of the boll on the plant. It should be pointed out, however, that the causes of seed diversity are not limited to these factors; on the contrary, they are to a strong degree related to individual environmental factors (water regime, mineral nutrition, etc.), and also to the interaction of the complex of these factors with the plant.

SUMMARY

The effect of the interaction between cotton plants and environment on subsequent germination of the seeds at comparatively low temperatures was studied. The experiments were carried out with 108-F and S-460 varieties of cotton. An analysis of the experiments led the authors to the following conclusions.

The development of cotton seeds to a large degree depends on temperature conditions during the growing period. The conditions largely determine the diversity of seeds in respect to response to temperature during germination.

In general, the physical and biological diversity of seeds is a distinct property of the plant. It is closely related to the nature of the plant and to the conditions which influence it.

The vital potential of the seeds decreases with late development of the bolls. The most viable in respect to germination and other physiological functions are seeds from the lower and middle parts of the plants.

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ACTIVITY OF THE LEAF APPARATUS IN FORMATION OF SEEDS IN SUNFLOWER

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At the present time we know comparatively little about the conditions by which seeds obtain substances coming from the leaves. Out of many aspects of this complex problem, in this paper attention is paid chiefly to the effect of the physiological state of leaves on seed formation.

In a previous paper [1], it was shown that the leaves of the middle nodes play the major role in supplying seeds with nutrient substances as compared with leaves of upper and lower nodes. Furthermore, the leaves of the upper nodes are even to some degree competitive with seeds, since they obtain part of the nutrient substances which are directed to the reproductive organs. In the present paper additional data on this problem is given, as a result of carrying out what we believe to be more comprehensive experiments. Furthermore, on the basis of experimental material characterizing the physiological state of leaves, a rational explanation of the different behavior of leaves of the upper and middle nodes is given.

The first experiments were carried out with two varieties of sunflower: Saratovskii Early, and No. 4966, planted on April 7, 1957, in clay containers with soil. The plants of the first variety flowered June 11-14, and of the second variety June 20-22. The main experiments were carried out from July 25 to August 5. The duration of experiments was brought to the minimum, so that when part of the leaves were removed (an operation which permits one to clarify the role of the individual leaves in seed nutrition), the remaining leaves could not reconstruct themselves and take on the function of the leaves which had been removed.

The main feature of these experiments was that the same plant was simultaneously both experimental and control. For this the stem together with the capitulum was cut with a razor blade almost to the root in two equal halves. Afterward, six leaves were left on each half, of which two were considered as leaves of the upper nodes, and the following four, of the middle nodes. Such a reduction in leaf mass should, in our opinion, have favored the clarification of the role played by the leaves of the various nodes in seed nutrition. A plant prepared for the experiment in this way is shown in Fig. 1. One half of this plant was untouched and served as a control. In the other half, the leaves of the upper or middle nodes, depending upon the experiment, were removed. The splitting of the plant was carried out one or two days prior to the experiment. The first sample of seeds for analysis was taken one or two days after the beginning of the experiment, the second, at the very end of the experiment. Seeds taken from outer rows of the head were analyzed for their content of dry matter and oil.

Because plants were grown in containers, their vegetative mass was comparatively small (height about 100 cm, total number of leaves 16-18) and the dimensions of their upper and middle leaves were not very sharply distinct, as can be clearly seen from Fig. 1. The experiment was done in duplicate.

In order to find out the effect of the splitting operation itself, as well as the possibility of translocation of nutrient substances from one half into another through the root-neck and root system, a fully controlled experiment was carried out. The results of this experiment are given in Table 1.

As can be seen from Table 1, the operation of splitting did not itself cause any noticeable differences either in accumulation of dry matter, or in the oil content of seeds in separate halves of the split stem. At the same time, the complete removal of leaves in one of the halves sharply decreased the weight and oil content of seeds on this half as compared with the corresponding control half of the stem. This has been confirmed by radioactivity of the seeds in two halves of the split sunflower stem, when the leaves of one of the halves were fed labeled carbon dioxide. Analysis has shown a high specific radioactivity (300 counts/minute/mg) of seeds in the treated half of the stem and only traces in the other half (16 counts/minute/mg). Thus, one should suppose that although translocation of substances has taken place through the root system from one half of the split stem into the other, it is still insignificant, and does not mask the effect caused by leaf removal. As a whole this agrees well with other data [2], in which it had been shown that in the sunflower the leaves supplied nutrient substances primarily to the seeds of those parts of the capitulum which are located directly above them.

All the above material supports the use of the method of plant splitting for solving questions concerning the role of the leaves of various levels in seed nutrition.

In the main experiments, two upper or two middle leaves were removed on one half of the split plants, and

in all cases two more lower leaves were left on the experimental half of the stem, these being necessary for the nutrition of the root system connected with this half. In all cases, the corresponding foliated half of the stem was used as a control. The comparative determinations of dry matter and oil content of seeds both before and after the experiment, as well as the changes of these indicators during the experiment, give an idea about the participation of one or another set of leaves in seed nutrition. The results of these determinations for two species of sunflower are given in Tables 2 and 3. Study of the tables convinces us that even when plants are carefully selected for similarity in their external characteristics, they are still greatly different from each other in internal qualities. One could judge this on the basis of the data comparing the yield of the control half of the stems in different plants. However, this circumstance did not have much significance during the experiments with split plants, as the two halves of the same plant were being compared in all cases.

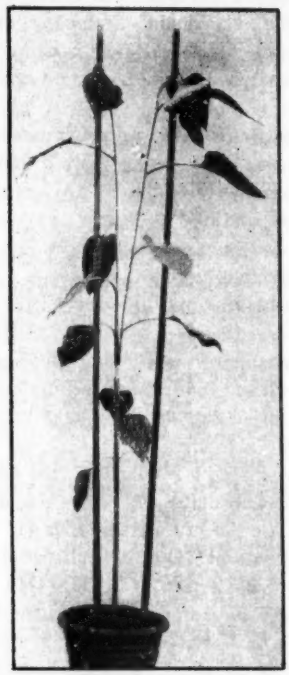

Fig. 1. Split sunflower plant during an experiment.

On the whole the data obtained clearly show the decisive role of the middle leaves in seed nutrition. Removal of the upper leaves in most cases either had no effect, or to a certain degree even increased dry weight and oil content of seeds. The latter may be explained by the fact that the upper leaves evidently absorb a part of the plastic substances from the general flow directed toward the seeds. Because of this, the removal of these leaves could under certain conditions favor the better nutrition, and consequently better growth, of the seeds.

The fact that the upper leaves absorb substances translocated along the stem in a much more intensive way than the other leaves, had been proven also in experiments with labeled substances. In these experiments the radioactivity of separate leaves of sunflower plants was compared, where labeled acetate (2.5 μC/ml) was applied by means of daily wetting of the upper surface of the eighth and ninth leaves for 4 or 5 days.

Radioautographs of the first, third, and seventh leaves, counting from above, are given in Fig. 2. As can be seen, the greatest radioactivity is shown by the uppermost leaf.

Beginning with the idea of the unity of the organism, it is natural to expect that in normally developing plants the functions of all organs in the period of seed ripening should be occupied by a single purpose—the provision of seeds with plastic and other necessary substances. It is obvious that the rate and direction of flow of these substances, as well as the activity of the corresponding organs, should be determined by the rate of metabolism and accumulation in reserve of these substances in seeds. However, one may artificially affect these processes—for example by removal of seeds or parts of seeds, which indicates a certain lability of these processes. This has been shown in the experiment with feeding of labeled acetate (the method is given above) to the middle leaves of normal and decapitated plants of sunflower during the period of seed ripening.

The results of this experiment are given in Fig. 3 as a radioautograph of leaves (the fourth from above) and young roots in decapitated (on the left) and normal (on the right) plants. From the radioautograph, it can be seen that the removal of seeds favors the flow of substances into roots and leaves, evidently in connection with the cessation of their use by seeds.

On the basis of study of enzyme activity and chemical composition of leaves of various levels in potato [3], oats [4], beet [5-7], etc., many authors also come to the conclusion that the leaves of the middle level have a very special role in seed nutrition.

One may suppose that the nature of differences in behavior of leaves of various levels consists above all in peculiarities of certain basic physiological processes occurring in leaves, photosynthesis, respiration and finally outflow of substances, as indicators of their role of supplier.

Below are given data characterizing the leaves of the various levels particularly from this point of view.

In these experiments several varieties of sunflowers were used—Saratovskii Early, No. 4966 and No. 8931, planted in the field. The determinations of respiration rate were carried out by the method described above [8], the photosynthetic rate, by means of Richter absorbers [9]. The amount of flow from leaves was represented by the difference between value of fall of the dry weight in darkened leaves on the plants, and cut leaves (method of Sachs). The darkening of the leaves was carried out

TABLE 1. The Effect of Splitting and Leaf Removal in One Half of the Stem on Comparative Accumulation of Dry Matter and Oil in Seeds in Both Halves of the Stem. Duration of the experiment, 10 days

Variety	State of both halves of split stem		Dry weight of 100 seeds, g		Oil content, %	
	1	2	1	2	3	4
Saratovskii Early	Foliated	Foliated	1.71	1.61	48.4	48.7
	Without leaves	The same	1.04	1.53	42.0	60.2
No. 4966	Foliated	The same	3.19	3.04	55.5	54.0
	Without leaves	The same	1.78	2.32	–	–

TABLE 2. The Effect of Leaf Removal of the Upper and Middle Levels on the Yield and Oil Content of Seeds in Saratovskii Early Sunflower (The experiment has been carried out with splitting of the plants from June 26 to August 5)

Foliation of the experimental half of the stem	Dry weight of 100 seeds (mg) in two halves of the stem						Oil content in 100 seeds (mg) in two halves of the stem					
	start of expt.		end of expt.		increase		start of expt.		end of expt.		increase	
	1	2	1	2	1	2	1	2	1	2	1	2
All leaves removed	0.36	0.41	1.04	1.53	0.69	1.12	0.13	0.15	0.43	0.92	0.30	0.77
Two upper leaves removed	1.04	1.14	2.59	2.54	1.55	1.40	0.42	0.49	1.29	1.26	0.87	0.77
The same	0.56	0.49	1.64	1.26	1.08	0.77	0.16	0.17	0.63	0.47	0.47	0.31
Two middle leaves removed	0.67	0.73	0.99	1.75	0.33	1.02	0.19	0.19	0.42	0.73	0.24	0.54
The same	1.64	1.38	1.94	2.14	0.30	0.76	0.82	0.83	1.02	1.23	0.20	0.40
The whole plant		0.98		1.95		0.97		0.39		0.77		0.38
The same		1.46		3.00		1.54		0.71		1.40		0.69

NOTE: 1) Experimental plants; 2) controls.

TABLE 3. Effect of Removal of Leaves of the Upper and Middle Levels on Yield and Oil Content in Seeds of Sunflower Variety No. 4966. Experiment carried out with split plants from June 26 to August 5

Foliation of the experimental half of the stem	Dry weight of 100 seeds (mg) in two halves of the stem						Oil content in 100 seeds (mg) in two halves of the stem					
	start of expt.		end of expt.		increase		start of expt.		end of expt.		increase	
	1	2	1	2	1	2	1	2	1	2	1	2
From all leaves	0.73	0.66	1.78	2.32	1.06	1.66	0.21	0.20	0.77	0.93	0.56	0.73
Two upper leaves removed	0.87	0.75	2.57	2.05	1.69	1.29	0.20	0.19	1.30	1.06	1.10	0.87
Two middle leaves removed	1.43	1.35	3.66	4.25	2.23	2.90	0.39	0.36	1.95	2.46	1.56	2.10
The same	0.85	0.80	1.98	2.58	1.12	1.79	0.22	0.21	1.03	1.38	0.81	1.17
The whole plant		1.28		2.71		1.43		0.40		1.22		0.82
The same		1.71		3.47		1.76		1.63		2.06		1.43

NOTE: 1) Experimental plants; 2) controls.

for three hours—from 12:00 noon to 3:00 PM. For determination of dry weight of leaves we cut corresponding discs 18 mm in diameter with a sharp instrument.

The results of determination of respiration rate of upper (2nd leaf from above) and middle (7th leaf from above) leaves are given in Table 4. As can be seen from

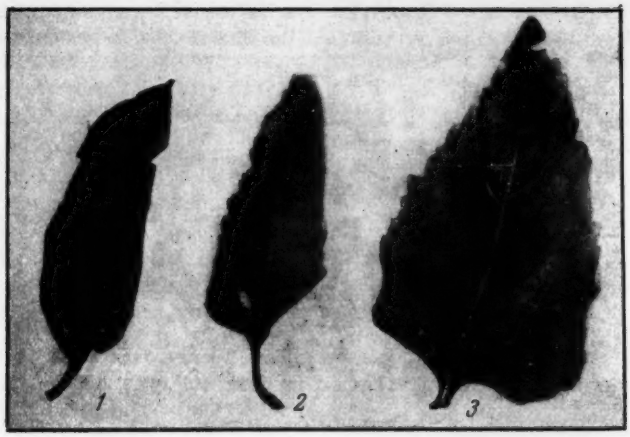

Fig. 2. Radioautographs of leaves (from left to right) of the first, third, and seventh leaf from above, in a sunflower plant fed with labeled acetate.

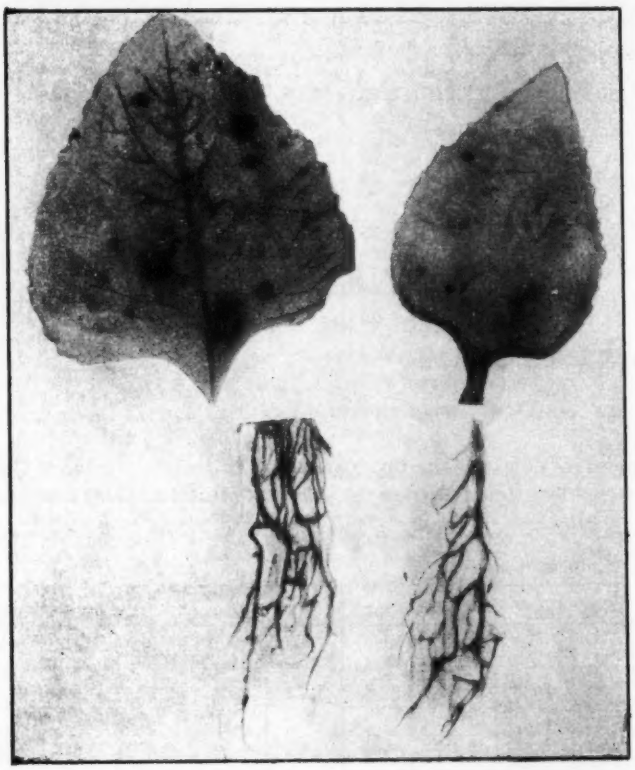

Fig. 3. Radioautograph of leaves (fourth from above) and young rootlets in decapitated (on the left) and normal (on the right) sunflower plants, fed with labeled acetate.

Table 4, in all varieties (in all age limits measured) the respiration rate of upper leaves is higher than that of the middle leaves.

The increase in respiration rate from lower leaves towards upper ones has also been noticed by other investigators [7, 10]. However, the leaf respiration rate cannot by itself give the proper picture of the participation of these leaves in seed nutrition. For this, it is necessary to compare this value with the indicator of photosynthetic rate.

Data characterizing the photosynthetic rate in leaves of the upper and middle levels in the morning

TABLE 4. The Respiration Rate of Leaves of the Upper and Middle Levels in Sunflower Under Field Conditions (In ml CO_2 evolved per hour, per gram of wet or absolutely dry weight). September 1956

Variety	Age of plants, in days after flowering	Respiration rate			
		Upper leaf		Middle leaf	
		wet weight	absolutely dry weight	wet weight	absolutely dry weight
No. 4966	30	7.4	32.8	4.1	18.2
	36	6.4	23.0	5.3	23.2
No. 8931	23	0.7	3.2	0.3	1.3
Saratovskii Early	33	1.9	8.0	0.3	1.5
	46	5.9	22.8	5.7	21.5

TABLE 5. Photosynthetic Rate of Leaves from the Upper and Middle Levels in Sunflower No. 4966 (In ml CO_2 per 100 cm^2 per hour). July 1956

Age of plants, in days after flowering	Photosynthesis of leaves	
	Upper	Middle
10	7.3	9.8
9	2.6	11.2
13	5.4	5.2
13	7.3	9.1
21	2.9	5.4
23	4.9	6.0
25	4.7	10.4
26	1.3	4.6

hours (from 8:00 to 11:00 AM) in plants of Sunflower No. 4966 under field conditions are given in Table 5.

From the data of Table 5, it follows that the photosynthetic rate of the upper leaves, as the reverse of respiration, is lower than in middle leaves. Analyses also have shown that the photosynthetic rate depends not only on the age of the leaf but on the total age of the plant, as can be seen from data of Table 6. In these experiments, the determination of photosynthesis in plants of different age was carried out in the same morning hour for three days, during which similarly warm and cloudless weather prevailed. The experiment included plants having a different number of days of ripening, beginning with butonization and ending 27 days after flowering, which corresponded approximately to the middle of the whole period of ripening. As can be seen from Table 6, the lower photosynthetic rate in leaves of the middle and upper levels was noted during butonization and the first days of the flowering.

In the literature, the question of the effect of leaf location on its photosynthetic activity is contradictorily described. Some authors insist that, from the moment of appearance of the leaf until its full development, the rate of photosynthesis at first grows, reaches a maximum, and then falls as the leaf ages [11, 12]. Finally, according to the data of Gorbuno [10] in experiments with different species of plants, in the middle phases of plant development the photosynthetic capacity of individual leaves increases from lower leaves up to the upper ones, where it reaches its maximum value, while the beginning and final phases of development are characterized by a greater rate of photosynthesis in the leaves of the middle levels, as compared with the upper and lower ones. It can be seen that, as a whole, this question is very complicated, and evidently cannot have the same solution in all cases. In our experiments, it was easier since the determination of the photosynthetic leaf activity was carried out only during a comparatively short period of seed ripening. This permitted a clearer establishment of the relationship between the leaf level and the amount of photosynthesis in leaves, ignoring the effect of the plant passing from one phase to another on this process.

On the basis of the data we obtained, we still may insist that the greatest photosynthetic rate during the period of seed ripening is shown by the leaves of the middle levels, as compared to the leaves of the upper levels.

During comparison of the values of respiration and photosynthesis rates, it could be said a priori that the upper leaves must play a considerably more modest part in seed nutrition than the leaves of the middle levels. This rule was shown very clearly during the study of the amount of flow of plastic substances in leaves of various levels. This rule was shown very clearly during the study of the amount of flow of plastic substances in leaves of various levels. The results of corresponding experiments are given in Table 7, where comparative figures are shown, characterizing the loss of weight in the darkened leaves, both those which were cut and those that are still on the plant. On the basis of the data of Table 7, it may be concluded that in the upper leaves, in most cases, not only was no flow observed, but, on the contrary, the absorption of plastic substances took place, which is indicated by the figures of a greater fall in weight of cut leaves (respiration) as compared with the intact leaves (respiration and flow).

Insofar as middle leaves are concerned, a considerable decrease of weight was noted, because of flow. In lower leaves, the flow was expressed very weakly.

Thus, the experimental results obtained are convincing in pointing out that the middle leaves are actually the suppliers during the period of seed formation, i.e., it is particularly from them that the nutrient substances flow into seeds and other organs of sunflower plant. At the same time, it is necessary to note that the limits between upper, middle, and lower leaves are not fixed precisely on the plant. Under the influence of various factors (density of stand, conditions of mineral nutrition, water supply, etc.), they may move up or down.

TABLE 6. The Rate of Photosynthesis in Leaves of Various Levels According to the Total Age of Plants in Sunflower No. 4966. (In ml CO_2 per 100 cm^2 per hour). August 1957.

Consecutive number of the leaf, counting from above	Number of days from the beginning of the flowering								
	Butonization	1	3	6	8	10	12	18	27
1-3	Respiration*	6.6	Respiration*	4.04	4.6	4.4	2.1	2.1	6.4
5-13	2.4	7.6	10.0	5.6	11.6	12.4	7.5	9.4	9.2
14-18	–	–	3.2	–	–	–	–	–	–

*Evolution of CO_2.

TABLE 7. Flow of Nutrient Substances from Leaves of Various Nodes, in Sunflower Saratovskii Early, Determined by the Sachs Method, and Expressed in mg per g Absolute Dry Weight of Leaf per 3 Hours (July-August 1956).

Number of days after flowering	Change in dry weight of darkened leaves								
	Upper			Middle			Lower		
	On plant	Cut	Flow	On plant	Cut	Flow	On plant	Cut	Flow
6	–41	–24	17	–55	–21	34	–17	–10	–7*
7	–68	–62	6	–71	—	—	–10	–37	6
7	–51	—	—	–110	–61	49	—	–56	—
9	–18	–120	–102*	–108	–33	76	–15	–27	–12*
11	–179	–171	8	–65	–80	–15*	–42	–15	27
12	–76	–117	–31*	–126	18	–108*	–16	–7	9
14	—	–103	—	–62	–9	52	–12	–5	8
16	–41	–87	–46*	–69	–49	20	–28	–66	–38*
17	–44	–123	–79*	–90	–38	52	–65	–84	15

NOTE: *Sign in the flow graph indicates not only absence of flow, but even absorption of plastic substances by a given leaf.

A natural development of investigations in this direction could be the study of the nature of substances flowing out of the leaves in general, and from leaves of various levels in particular. There is no doubt that carbohydrates are not the only products of synthetic activity of leaves, which furnish the nutrition of heterotrophic organs. It is known that substances causing flowering of plants are formed in illuminated leaves. These substances then flow into the stem growing point, where they stimulate formation of reproductive elements. Finally, Dostal [13], in his paper speaks of the idea that various products of photosynthesis are formed in the leaves of various levels.

SUMMARY

[1] For the study of the effect of leaf foliation on the quality of seeds we used the method of splitting of capitulum and stem of sunflower in two equal halves, one of which was experimental, and the other a control.

[2] By this method the leading role of middle leaves in seed nutrition was clearly shown, and in addition, in some cases even the fall of weight and oil content of seeds as affected by upper leaves. The latter evidently take from the common flow a part of the nutrient substances which flow from the leaves below towards the seeds.

[3] During the period of seed ripening the respiration rate of individual leaves increases from lower levels to the upper ones. The rate of photosynthesis was greatest in leaves of the middle level. The intensity of photosynthesis was greater in the period of seed ripening as compared with the previous period of butonization.

[4] Comparison of the value of flow from the leaves of various levels has shown that this value is greatest in the leaves of the middle level. Leaves of the upper level in most cases gave nothing, but on the contrary, absorbed nutrient substances themselves.

[5] The indicated interrelationships between leaves and seeds are observed in normal plants during the period of rapid seed ripening. However, as a result of lability of processes in the growing organisms these interrelationships may be changed under the influence of various factors. An example of this may be the phenomenon of change in direction of the flow of nutrient substances as a result of decapitation.

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EFFECT OF SEED HYDRATION ON MOBILIZATION OF RESERVE SUBSTANCES DURING GERMINATION

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In numerous papers [1-11] devoted to the study of transformation of reserve substances during germination of seeds of different plants, certain important points have been established. Thus, for example it has been shown that, as a result of mobilization of reserve fats new carbohydrates appear, which flow to the growing parts of the seed. The reserve protein breaks down to free amino acids, which are then transformed into constitutional proteins of the seedling. However, many aspects of metabolism of germinating oil bearing seeds remain unclarified.

In a previous paper [11], we suggested that the rate of mobilization of reserve substances depends on the degree of hydration of tissues. Thus, for example a more rapid utilization of fats was observed in those sunflower seeds with greatest moisture content. Supposing that the moisture of seeds may change not only the rate of mobilization, but also its nature, we have tried experimentally to show how mobilization of such reserve substances as fat, proteins and carbohydrates is changed in relation to seed hydration. We have studied the effect of three different values of seed moisture: 20-26, 31-40, and 42-50%. Certain variations in seed hydration were conditioned by the fact that the experiments were carried out in an atmosphere saturated with water, as a result of which a certain increase in seed hydration took place during an experiment. To save time, we shall refer to these levels of hydration hereafter as 20, 30, and 40%. In order to avoid the effect of microorganisms, the experiments were carried out under sterile conditions.

Seeds of sunflower, variety Saratovskii 169, were taken as experimental material. Before the experiment, the seed-coats were removed and the embryos were sorted by weight on a torsion balance, within limits of 2 mg. After sorting, the embryos were sterilized with 10% hydrogen peroxide. The duration of sterilization was varied according to the degree of seed hydration. "Dry" seeds, with a moisture of about 4%, were sterilized for 12-15 minutes, while swollen seeds were treated for 5-6 minutes, followed by washing with sterile water. Seed sterilization was done in a conical flask, after which the seeds were transferred aseptically into sterile 200-250 mm test tubes for further cultivation. For cultivation of seeds with 30-40% moisture, 3 ml of water were placed at the bottom of the test tube. Since a great

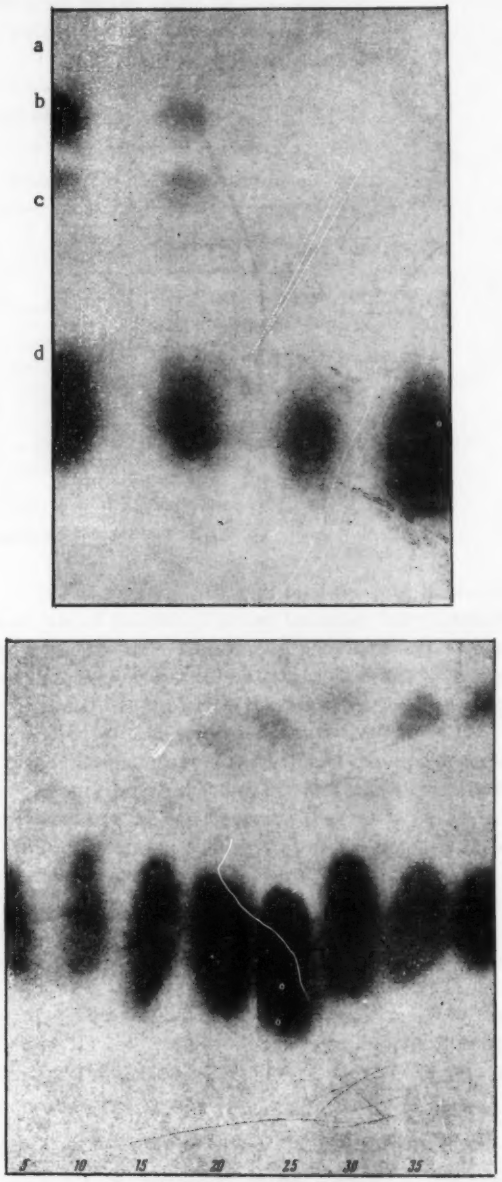

Fig. 1. Descending chromatogram of carbohydrate content in Cotyledons, at 40% moisture.

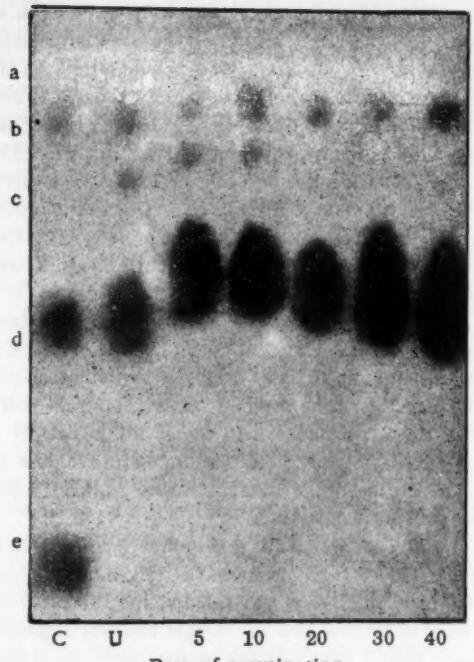

Fig. 2. Descending chromatogram of carbohydrate content in cotyledons, at 30% moisture. C) Controls; U) ungerminated seeds.

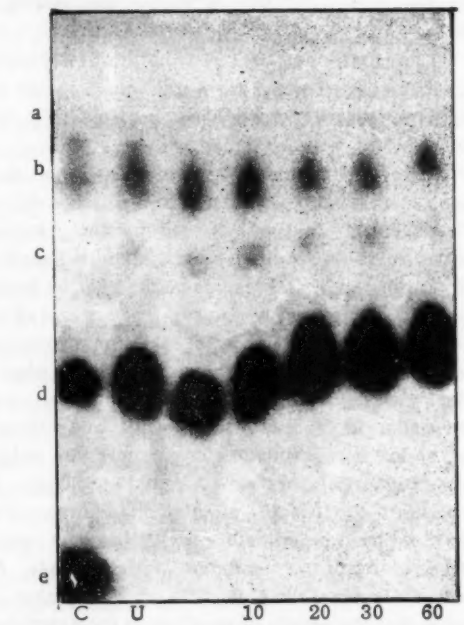

Fig. 3. Descending chromatogram of carbohydrate content in seeds at 20% moisture. C) Controls; U) ungerminated seeds.

decrease in seed moisture was observed in the absence of the free water. Test tubes were placed in a desiccator in which 100% relative humidity was maintained. The air in the desiccator was kept free from the carbon dioxide produced by the seeds by absorption with potassium hydroxide. Desiccators were placed into an incubator with a temperature of 26°. Different moisture levels of seeds were achieved by varying the duration of soaking. For hydration of seeds up to 20-26% moisture, it was sufficient to hold seeds in hydrogen peroxide solution for 13 minutes (time of sterilization) and 7 minutes in the wash water. 31% moisture was reached by soaking seeds for two hours. For hydration of seeds up to 42%, they were soaked for twenty-four hours. Material was fixed by dehydrated oil, since comparative analyses have shown that fixation with steam and dehydrated oil give the same results. Material was dried to constant weight at 95%. After drying, determination of the fat content of cotyledons was done by the method of Rushkovskii [12]. Fat was extracted by petroleum ether, with a boiling temperature of 50-60°. The free amino acids and carbohydrates were extracted from the defatted material by the method described in the previous paper [11]. Determination of carbohydrates was carried out by descending chromatography, the sugars being developed by the reagents proposed by Boyarkin [13]. Free amino acid content was indicated by ascending chromatographs, developed with isatin [13].

It can be seen from the data shown that no growth of seedlings was observed at 20% seed moisture. At 30%

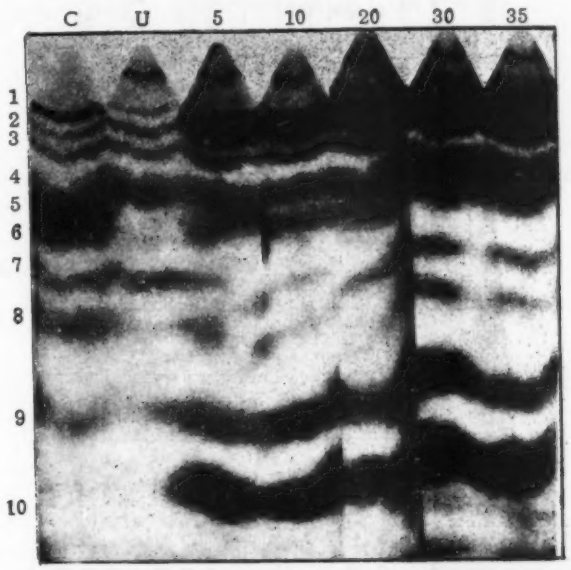

Fig. 4. Ascending chromatogram of free amino acid content in cotyledons at 30% moisture. Numbers on separate chromatograms show duration of germination of seeds, in days; numbers on the side stand for amino acids: 1) Histidine; 2) arginine; 3) asparagine; 4) aspartic acid; 5) serine; 6) glycine; 7) glutamic acid; 8) threonine; 9) alanine; 10) proline.

TABLE. Change in Seed Composition During Germination in Relation to the Degree of Seed Hydration

Duration of germination, days	20–25%						30–40%						40–50%					
	moisture, %	wt. of 1000 seeds, g	wt. loss, g	% oil	wt. of oil in 1000 seeds, g	Loss of oil, g	moisture, %	wt. of 1000 cotyledons, g	wt. of 1000 seedlings, g	wt. loss, g	% oil	wt. of oil in 1000 seeds, g	Loss of oil, g	moisture, %	wt. of 1000 cotyledons, g	wt. of 1000 seedlings, g	Loss of oil, g	% oil	wt. of oil in 1000 seeds, g	Loss of oil, g
Beginning	23.0	33.2	—	54.9	18.23	—	31.0	37.6	37.6	—	56.9	21.39	—	43.9	45.7	45.7	—	57.4	26.23	—
5							31.0	33.8	36.5	1.10	57.1	19.29	1.10	45.5	38.5	44.5	1.2	55.6	21.40	4.83
10	20.6	33.2	0.00	54.8	18.19	0.04	30.9	32.2	35.6	2.00	58.0	18.68	2.71	43.2	38.5	44.2	1.5	53.2	20.48	5.75
20	22.8	32.8	0.40	54.4	17.84	0.39	40.9	31.3	34.9	2.70	53.2	16.65	4.74	50.6	34.8	41.7	4.00	48.4	16.84	9.39
25																				
30	23.5	32.5	0.70	54.6	17.74	0.49		28.5	33.2	4.40	51.4	14.64	6.75	50.2	33.5	39.7	6.00	44.4	14.87	11.36
35							41.3													
60	25.8	31.9	1.30	52.8	16.84	1.39														

and 40% seed moisture, the growth of seedlings was retarded as compared with growth at greater values (57-60%) of cotyledon moisture.

In the latter case the weight of the seedlings increased 10-12 g in 15 days, while at 30% moisture the seedling weight increased only 4.7 g in 35 days, and 6.2 g at 40% moisture. In spite of the absence of any visible growth, or growth retardation, the mobilization of reserve substances takes place in cotyledons. At 20% seed moisture, a gradual decrease of absolute fat content is observed in seeds (see Table). Thus, out of 18.23 g of fat, in 1000 seeds, a decrease of 1.39 g was observed in 60 days, making an 8% decrease. One should note that, at this moisture value, only after prolonged cultivation (60 days) does one observe an excess of fat loss over the loss in the dry weight of seeds.

At 30% seed moisture, the decrease of fat content takes place faster than at 20% moisture. Thus, 6.75 g of fat was used up in 35 days of cultivation which is approximately 30% of the original fat content. The loss of fat at a given moisture value exceeds the loss of dry weight, from the 10th day of cultivation. With increase in cultivation time, the difference between fat loss and dry weight loss is increased.

Mobilization of fat takes place considerably more intensively at 40% moisture. At this value, the amount of fat lost in 35 days was 11.35 g, i.e., about 43%. The excess of fat loss over dry weight loss was observed already after 5 days. As can be seen from the table, the difference between fat loss and dry weight loss in seedlings is increased with increase in seed moisture. If at 20% moisture the difference between fat loss and dry weight loss was 0.09 g in 60 days, then at 30% seed moisture, the value was 2.35 g in 35 days, and at 40%, it was 5.36 g.

The excess of fat loss over the loss of dry weight indicates that fat is transformed into other organic substances. With greater seed moisture, evidently, better conditions for fat transformation exist.

As can be seen from the data of the table, the sprouting of seedlings is related to the loss in dry weight, independent of the degree of seed moisture. Increase in the dry weight of seedlings from oil-bearing seeds which are germinated in darkness, as pointed out by Barmenkov [5], is, in our opinion, a result of inaccuracies of method related mainly to the incorrect selection of material for analysis. As is well known, plant seeds show great individual variation even within limits of one fruit, not even mentioning the seeds of different fruits and different plants. In our experiments, for example, the weight of embryos of a sunflower varied from 30 to 90 mg. Since seedlings of small seeds grow sick and die more often than those from large seeds, every subsequent sample may be enriched more and more by large seeds. As a result, there arises the possibility of the mistaken conclusion that the weight of seedlings is increased during germination of oil-bearing seeds, as compared with the weight of the seeds themselves.

The correct solution of this problem also depends on the method of seed analysis. Prolonged drying of the seeds at high temperature may be accompanied by oxidation of oil and increase in the weight of the material.

Thus, in spite of the fact that transformation of fat into carbohydrates and other products takes place similarly in the seeds of different weight and is accompanied by addition of water and oxygen, i.e., leads to an increase in biomass, the general balance of dry matter in germinated seeds results in a deficit of dry weight. Evidently, the expenditure of substances for respiration prevents increase of weight in products, which would have been conditioned by transformation of the methyl and methylene groups of fat into the hydroxyl and carboxyl groups of carbohydrates, acids, and other products.

Carbohydrate Utilization. As data of the chromatographic analysis show (Figs. 1, 2, 3), the sequence of oligosaccharide utilization changes in relation to the degree of seed hydration. Thus, at 40% seed hydration (Fig. 1), as we have already noted [12], the first to be utilized are the high-molecular weight oligosaccharides a, and then the less complex ones b and c. In seeds of this moisture content, the times of utilization of different oligosaccharides are very close together, and by the third day of cultivation all three groups of oligosaccharides are being utilized. After 25 days of cultivation, oligosaccharide b appears again. At 30% seed moisture, the time of mobilization is prolonged, and sequence of oligosaccharide utilization changes.

As in the case of 40% moisture, the first to be utilized is oligosaccharide a, which is absent within two hours after soaking (Fig. 2, ungerminated), then after 20 days, oligosaccharide c disappears. Oligosaccharide b stays unutilized for 40 days, although by this time, about 30% of the fat has already been utilized.

At 20% seed moisture, a simultaneous decrease in all three groups of oligosaccharides takes place (Fig. 3). However, their utilization takes place so slowly that their content is only insignificantly decreased even after 60 days. The presence of oligosaccharide b in cotyledons at 30% moisture, and its appearance after 25 days of cultivation of seeds with greater moisture content (40%), gives us a basis for suggesting the possibility of formation of this product from fat. This takes place, evidently, when the processes of mobilization take place with greater intensity as compared with utilization of hydrolysis products for growth processes. As was pointed out above, the growth of seedlings was very retarded in our experiments. The disappearance of oligosaccharide b during the first three days in cotyledons with 40% moisture is evidently connected with its utilization for growth processes. At a later time, water uptake is retarded and growth processes are slowed down, while hydrolytic processes evidently occur at the same rate. As a result of lack of correspondence of hydrolytic processes with those of growth, oligosaccharide b accumulates, either as a product of fat breakdown or through resynthesis. Sucrose was present in cotyledons throughout the whole period of cultivation, independent from seed moisture. The absence of monosaccharides in cotyledons seems to indicate their rapid transformation.

Protein Mobilization. As was indicated above, we estimated the mobilization of protein according to the amount and number of free amino acids. At 20% seed moisture, we could find no significant changes in free amino acid content. With seed hydration up to 30% and 40%, a rapid increase in the amount of present amino acids takes place, as well as the appearance of new ones, such as glycine, threonine, aminobutyric acid, tyrosine, methionine, valine, phanylalanine and leucine. It is interesting to note that in contrast to other amino acids, the content of which noticeably increases as germination takes place, the amount of glutamic acid changes haphazardly: Either it greatly decreases (Fig. 4), or again it reaches the original value. Evidently this indicates a closer connection of glutamic acid with growth processes, which are retarded in the later period of cultivation.

SUMMARY

1. The rates of mobilization of reserve substances in sunflower seeds depend on the degree of seed hydration. Already at 20% moisture content, a slow mobilization of reserve products was observed, which increased with the degree of seed hydration. Degree of seed hydration determines not only the rate of mobilization but also the sequence of oligosaccharide utilization.

2. At 40-60% seed moisture, growth processes are retarded, and losses of fat exceed the decrease in dry weight of seeds, which indicates transformation of part of the fat into other organic substances. In the case of greater hydration (70%), when growth processes take place energetically, a similar phenomenon is not observed.

3. With increase of hydration of germinating seeds, the number and amount of free amino acids in the seedling is increased.

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SOME PECULIARITIES OF WATER ABSORPTION BY MAIZE SEEDS

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The absorption of water by seeds is one of the essential features of their growth. A great many studies have been made on the water relations of seeds. However, many special features of the process of water absorption still remain inadequately explained. A more thorough knowledge of this process has great practical significance, since a deviation within the water relations of seeds from the optimum is one of the frequent causes of a decrease in their field germination, and irregular and prolonged germination; this results in unevenness of the vegetative stand and a decrease in yield.

As one of the inadequately studied special features of water relations in seeds we should consider the problem concerning the activity of the embryo in the absorption of water. Furthermore, unless the role of the embryo is properly represented one cannot fully understand the process of seed germination and its relationship with external factors. Sometimes this problem is generally not touched upon and the swelling of the seeds is considered as a single process [1-5].

The lack of study of this problem is largely explained by the difficulties encountered in the procedures for setting up the necessary experiments since the seeds represent a single structure whose parts are all closely mutual ly related physiologically. Therefore, it is technically quite difficult to separate the activity of the individual parts of the seed in the absorption of water, and unfortunately conclusions must be drawn primarily on the basis of indirect inferences and not on the basis of direct experimental data; for example, the comparison of water absorption by part of a wheat seed with the embryo and without the embryo [1, 6]. In some experiments the participation of the embryo in water absorption was eliminated by narcotizing the seeds with ether [6], chloroform [7], or killing them with toluene [6] and assuming that in such a case the absorption of water would be determined by colloidal properties of the seed. The difference in swelling between the controls and the treated seeds should indicate the participation of the embryo in the absorption of water. However, seeds which have been narcotized or killed with narcotics may still contain active enzymes and during swelling it is possible that osmotic forces may appear as the result of hydrolysis of substances stored in the seed; this would be due to the activity of the living part of the seed which has been activated by the incorporation of water in preparation for germination even though the embryo itself is not alive. When the living parts of the seed are killed by high temperature there occurs a serious change in the properties of many of the substances; this prevents transformation of substances and the absorption of water which, in this case, would be due to the elimination of embryo activity.

It seemed more correct to resort to a mechanical elimination of the embryo and the scutellum, or the embryo only during an investigation of the significance of the activity of the living parts of the seed in the process of water absorption. In such a case, swelling of the remaining parts of the seed would be determined primarily by physico-chemical forces, whereas in seeds with an embryo there exist closely interwoven forces of a different nature. By comparing the course of swelling in seeds with an embryo and in those without an embryo one can show the special features of water absorption which are due to the activity of the embryo.

Experiment No. I was set up on the basis of this method.

Intact and operated maize seeds, variety Minnesota 13 extra, were weighed individually on a torsion balance. The water content was determined in a parallel portion of seeds. The weighed seeds were immersed in tap water in a ratio of water: seeds = 20 : 1 at a temperature of 22-23°. After a designated time interval the seeds were removed from the water, their surface dried with filter paper, weighed on the same balance, and again immersed in fresh water until the next weighing. There were 20 seeds in each part of the experiment.

Even after 23 hours from the onset of swelling there was a difference in the amount of water absorbed by the seeds of each group (Table 1). Whereas one seed without an embryo absorbed an average of 113.5 mg of water, which represented 49.9% of its absolute dry weight, one seed with an embryo absorbed 136.1 mg of water, and its water content reached 53.0% of the dry weight even though the seed without the embryo had an open "wound" and a greater absorbing surface because of this. A variation of 3.1% is statistically significant. There was practically no further entrance of water into the seeds without an embryo, and during subsequent weighings their weight varied within the limits of accuracy in the determination of weight increase. In seeds with an embryo, water continued to enter throughout the duration of the experiment (92 hours).

TABLE 1. The Course of Water Absorption by Maize Seeds (amount of absorbed water in mg and in % on the basis of initial absolute dry weight of the seeds)

Experimental variant	Beginning absolute dry weight of seeds, mg	Hours from beginning of swelling									
		Start of experiment		23		47		71		92	
		mg	%	mg	%	mg	%	mg	%	mg	%
Seeds without embryo	227.3	24.9	10.9	113.5	49.9 ± 0.45	119.4	51.3 ± 0.55	118.9	52.3 ± 0.58	117.5	51.7 ± 0.62
Seeds with an embryo	257.4	25.0	9.7	136.1	53.0 ± 0.85	156.1	60.3 ± 0.93	166.1	64.0 ± 0.93	169.3	65.5 ± 0.92
Difference in relation to seeds with an embryo		+0.1	−1.2	+22.6	+3.1	+36.7	+9.0	+47.2	+11.7	+51.8	+13.8

TABLE 2. The Course of Water Absorption by Maize Seeds (amount of water in % on basis of absolute dry weight of the seed)

Experimental variant	Hours from the beginning of swelling	Hours from beginning of swelling						
		3	5	7	14	18	21	24
Seeds without embryo	10.9	19.1	22.5	26.2	31.3	31.7	32.2	31.4
Seeds with embryo	9.7	12.2	15.7	19.5	29.2	31.2	33.5	33.0
Difference in relation to seeds with embryo	−1.2	−6.9	−6.8	−6.9	−2.1	−0.5	+1.3	+1.6

After 92 hours the endosperm of the seeds with an embryo contained 165.6 mg water, or 64.8% on the basis of its dry weight, and that of seeds without an embryo contained 117.5 mg water, or 51.7%. Therefore, the presence of the embryo increased the accumulation of water by the seed endosperm.

Under the conditions of the experiment, only during the first 23 hours did the water content of the seeds increase as the result of colloidal swelling. Later because of a change, or as a supplement to this process, the absorption of water (since substances of a colloidal nature are also present in the scutellum and in the embryo) occurred by another process which depends on the activity of the embryo.

In order to determine the time when the activity of the embryo began to have an effect on the absorption of water more accurately than in Experiment I, Experiment II was set up in the same way with shorter time intervals between weighings during the beginning of swelling (Table 2).

Experiment II showed that during the first hours water entered more rapidly into the seeds without an embryo; this might be expected, taking into account the presence of the wound. But after 21 hours the entrance of water into seeds with an embryo began to exceed the entrance of water into seeds without an embryo. If we assume an increase in the amount of water absorbed between the two weighings, one can see that even after 9-11 hours the seeds with an embryo began to absorb water more rapidly than the seeds without an embryo. These facts can be interpreted as an indication of the appearance of an embryo effect on the rate of water entrance, but the comparative acceleration of water entrance can also be explained by the fact that seeds with an embryo absorbed less water than seeds without an embryo, and then the entrance of new portions of water depended on the amount of water absorbed earlier [2, 6]. After the 21st hour the difference in water absorption can be explained only by the activity of the embryo.

The method of setting up Experiments I and II did not permit a separation of water entrance as the result of swelling forces from that which resulted from the activity of the embryo, since the difference in water entrance might occur as the result of the resistance of fruit and seed coats in seeds with an embryo, whereas in seeds without an embryo they were partially removed.

This gave rise to the necessity of setting up Experi-

TABLE 3. The Course of Water Uptake by Maize Seeds in Relation to Their Position and Temperature (amount of absorbed water in % initial weight of seeds)

Temperature °C	Position	Hours from beginning of swelling								
		7	24	31	48	72	96	120	168	192
21	Upward with embryo	9.2	19.0	21.0	24.8	30.8	32.9	Germinated		
	Upward without embryo	10.0	17.0	18.1	20.3	21.3	21.8	–	–	–
	Difference	−0.8	+2.0	+2.9	+4.5	+9.5	+11.1	–	–	–
	Downward with embryo	9.7	20.6	23.2	27.4	32.2	Germinated		–	–
	Difference between that and embryo upward	+0.5	+1.6	+2.2	+2.6	+1.4	–	–	–	–
13	Upward with embryo	–	16.0	–	22.5	25.1	29.1	30.2	–	–
	Upward without embryo	–	16.7	–	21.5	21.9	21.9	21.9	–	–
	Difference	–	−0.7	–	+1.0	+3.2	+7.2	+8.3	–	–
	Downward with embryo	–	18.8	–	27.5	30.3	34.5	37.4	–	–
	Difference between that and embryo upward	–	+2.8	–	+5.0	+5.2	+5.4	+7.2	–	–
7	Upward with embryo	–	16.9	–	21.6	25.0	26.7	28.1	36.6	40.7
	Upward without embryo	–	24.5	–	29.0	30.6	31.1	32.1	33.3	33.3
	Difference	–	−7.6	–	−7.4	−5.6	−4.4	−4.0	+3.3	+7.4
	Downward with embryo	–	17.3	–	22.0	25.1	25.9	27.9	37.5	41.6
	Difference between that and embryo upward	–	+0.4	–	+0.4	+0.1	−0.8	−0.2	+0.9	+0.9

ment III in which the seeds were prepared just as they had been in previous experiments but which were placed either embryo upward or downward on moist filter paper or sand. In addition, the seeds were placed under different temperatures since the embryo activity of maize is strongly related to temperature. There were 15 seeds in each variant. Periodic weighings were shortened either to the time the radicle appeared, or when subsequent weighings did not reveal a further increase in weight.

When the seeds were arranged with the embryo upward the absorption of water in both groups occurred under almost the same conditions through morphologically and anatomically identical surfaces of the seed which were located opposite the embryo.

If one examines the process of water absorption at 21° and at 13°, i.e., temperatures at which the embryo passes rapidly to the active stage, one can see that at the beginning water entered the seeds without an embryo more rapidly, or at the same rate, than it did into seeds with an embryo. However, after 24-48 hours the seeds with an embryo had begun to absorb more water and the difference changed from a negative to a positive one (Table 3). A different course was observed at a temperature of 7°. In this variant the water in the seeds with an embryo began to enter more rapidly only after 168 hours after swelling. This difference in the rate of water entrance into seeds with an embryo and into those without an embryo at various temperatures can be explained as the result of embryo activity which at 21° became less energetic than after 24 hours from the beginning of swelling, and began to have an effect on the further entrance of water into the seeds. At 13° embryo activity was less rapid and this effect on water absorption really appeared after 48 hours, but at 7° the effect of the embryo was observed only after 168 hours (Table 4).

It is necessary to note that at 7° the absorption of water by seeds without an embryo ceased 72 hours after swelling; in seeds with an embryo it increased noticeably after 120 hours following swelling when embryo activity, even though weak, began.

The position of the seeds during swelling (embryo upward or downward) had a different effect on the absorption of water in relation to the temperature at which swelling occurred. At 21°and 13° the seeds absorbed water more rapidly if the embryo was placed downward. At 7° the position of the seeds had no effect on the course of water absorption. Such a relation between the course of water absorption and temperature as well as seed position can therefore be explained as the result of embryo activity on water uptake by the seeds. Our data differ from the results of Davis and Parker's experiments [8], in which the maize seeds placed embryo downward absorbed more water at all temperatures than those placed embryo upward.

Energetic activity of the embryo begins after the seeds have reached a certain degree of swelling. In order that the embryo reach the condition at which it begins to exert an effect on the absorption of water, maize

TABLE 4. The Course of Increase in Water Content of Maize Seeds After 24 Hours (in % initial weight)

Temperature °C	Presence of embryo	Period (in hours) from the beginning of swelling							
		0-24	24-48	48-72	72-96	96-120	120-168	168-192	192-216
21	With embryo	19.0	5.8	6.0	2.1	–	–	–	–
	Without embryo	17.0	3.3	1.0	0.5	–	–	–	–
13	With embryo	16.7	7.4	2.8	5.8	1.6	–	–	–
	Without embryo	16.1	5.4	0.4	0	0	–	–	–
7	With embryo	16.9	4.7	3.3	1.8	2.1	7.7	4.2	9.1
	Without embryo	24.5	4.5	1.6	0.5	1.0	1.2	0	0

TABLE 5. The Change in Water Content of Individual Parts of Maize Seeds Depending on the Degree of Swelling at 22° (amount of water in one seed in mg and % of previous period)

Parts of seed	Hours after beginning of swelling								
	0	3		7		16		20	
	mg	mg	%	mg	%	mg	%	mg	%
Entire seed	29.73	49.58	166	67.0	135	92.8	138	100.3	108
Endosperm	27.40	42.50	155	56.5	133	70.0	124	75.4	108
Scutellum	2.00	6.70	335	8.4	141	20.7	220	22.0	106
Embryo	0.33	0.38	115	1.1	289	2.1	191	2.9	138

TABLE 6. Distribution of Absorbed Water Among the Individual Parts of Maize Seed at 22° (in % total water in seed)

Parts of seed	Hours after beginning of swelling						
	0	3	7	16	20	24	46
Endosperm	92.2	85.7	84.3	75.4	75.2	76.0	61.7
Scutellum	6.7	13.5	14.0	22.3	21.9	19.7	20.9
Embryo	1.1	0.8	1.7	2.3	2.9	4.3	17.4

TABLE 7. The Course of Water Absorption by Individual Parts of Maize Seeds at 22° (in % absolute dry weight)

Parts of seed	Hours after beginning of swelling						
	0	3	7	16	20	24	46
Entire seed	12.7	20.4	30.1	39.2	41.8	52.4	63.9
Endosperm	12.9	18.6	26.8	33.0	36.1	42.6	43.1
Scutellum	10.4	27.8	49.1	91.6	86.1	120.1	142.9
Embryo	9.6	10.1	43.6	102.5	112.0	126.7	511.2

seeds variety Minnesota 13 extra, must assimilate an amount of water equal to about 19-20% of their initial weight with a moisture content of 10-11%, this constitutes 33-34% of the absolute dry weight. For germination the water content of the seeds must reach 50% of their absolute dry weight. Therefore, of the 50% water which was necessary for the beginning of sprouting in maize seeds, 10-11% was found in air-dry seeds, 23-24% was absorbed as the result of the swelling of colloids in the seed, and the remaining 16-17% entered after the embryo had been aroused to activity.

Seeds without an embryo absorbed less water at all temperatures than seeds with an embryo. Apparently the absorbed water at certain periods of swelling is variously distributed among the main parts of the seed. In order to clarify the course of water distribution in relation to the degree of seed swelling, Experiment IV was set up.

Maize seeds variety VIR-42 were placed in a growth chamber between two layers of wet filter paper and transferred to various temperature conditions. There were 15 seeds in each variant. Seeds were removed from the growth chambers periodically, their surfaces dried with filter paper, and each seed was weighed on an analytical balance. Then, using a binocular lens, the seeds were rapidly separated into three parts—endosperm, embryo, and the scutellum, which is quite large in maize, and occupies a considerable area in the seed. The parts of each seed were placed individually into glass weighing flasks and weighed in the fresh and the dry state. The amount of water was expressed in milligrams and in percentage of absolute dry weight of the given seed part. The total amount of water in the seed was calculated by totaling the water in the individual parts. The dry weight was calculated in the same way.

The distribution of water in maize seeds in relation to the degree of swelling at 22° is shown in Tables 5, 6, and 7.

During the first three hours of swelling a large part of the water entered the endosperm, but during this peri-

od the comparative amount of water increased most noticeably in the scutellum. Provided the water content of the endosperm had increased from 12.9% of the dry weight to 18.6% in three hours, then the moisture in the scutellum had risen from 10.4 to 27.8%, i.e., the amount of water in the endosperm increased one and one-half times whereas in the scutellum it increased 3.3 times. The moisture content of the embryo had not changed within this period, or else this change was not detectable by the method used.

Such a distribution of water corresponds to that value which the individual parts of the seed have. During the preparation of maize seed for germination, as nearly as one can tell from the distribution of absorbed water, the scutellum becomes activated earlier than the other parts of the seed.

After seven hours the amount of water in the embryo had increased almost threefold compared with the amount present during the previous period. The hydration of the scutellum also increased very sharply. Water also continued to enter the endosperm, but the comparative increase of its hydration was not great, and after 16 hours of swelling the endosperm had practically reached saturation. From that time on water moved primarily into the embryo. It is true that the actual amount of water in the embryo is insignificant because it is small, however, for physiological performances of special occurring processes the relative water content, or hydration of the organs or tissues, is more important than the actual amount of water present in them. After 20 hours of swelling, hydration of the embryo began to exceed hydration of the other seed parts and rapidly increased with further swelling. After 46 hours it exceeded 500%. Within this time the portion of water in the endosperm was less than two-thirds of all the water absorbed by the seed, one-fifth was concentrated in the scutellum, and the remaining 17.5% was in the embryo.

TABLE 8. The Change in Dry Weight of Individual Parts of Maize Seed Depending on Their Degree of Swelling at 22° (in mg per single grain)

Parts of seed	Hours after beginning of swelling				
	0	3	7	16	20
Endosperm	212.4	214.4	211.1	211.8	209.1
Scutellum	18.7	19.2	19.7	22.6	25.4
Embryo	3.6	3.0	3.1	2.1	2.6

As the water was absorbed and the germination processes developed in the seed, a redistribution of organic substances began which manifested itself in a change of dry weight of the individual parts (Table 8).

During the first 20 hours of swelling the weight of the scutellum increased somewhat and the weight of the endosperm decreased. The weight of the embryo did not change, and perhaps even decreased somewhat as the result of the loss of materials due to respiration. However, this requires experimental verification.

Using the method described, the course of water absorption by individual parts of maize seeds at a temperature of 7-8° was also studied (Tables 9 and 10).

The absorption of water by maize seeds and their individual parts at this temperature had the same general nature that was noted at a temperature of 22°, but it occurred considerably more slowly. Therefore this discrepancy in water uptake was due primarily to the scutellum and the embryo. For example, at 22° and after 48 hours, the endosperm had taken up an amount equal to 43.1% of its dry weight, but at 7° it had taken up 39.2%. During this time the scutellum had absorbed an amount corresponding to 142.9 and 91.0%, and the embryo, 511.2 and 144.0%. Thus, during swelling at a temperature of 7°, i.e., a temperature at which maize seeds do not germinate, after five days all the seed parts, except the embryo, had reached the saturation at which the seed germinates.

After 120 hours of swelling at 7° the dry weight of the individual seed parts had practically not changed. Thus, the weight of the scutellum increased from 23 mg (which it weighed after six hours of swelling) to 26.6 mg, and the weight of the embryo increased from 2.1 to 2.3 mg. The absence of change in the weight of the scutellum and the embryo when swelling occurred at 7° indicates an inhibition of the translocation of metabolites between the endosperm, embryo, and scutellum, which had been observed at 22°. From this one can see the basic difference in processes which occur in seeds when they germinate at low temperatures. At a higher temperature—one optimal for germination—not only does water absorption occur but also the translocation of organic substances from the endosperm into the scutellum and the embryo; at a lower temperature only water absorption occurs but there is not translocation of substances, i. e., there is no embryo growth but merely water saturation and swelling. The latter was noted earlier by T. Z. Krasnosel'skaya-Maksimova [7].

A simultaneous calculation of the course of water absorption and the change in dry weight of the individual seed parts made it possible to calculate the relationship of water absorption on the basis of the dry weight of the embryo. It appeared that during the first 24 hours of swelling at 22° the amount of water absorbed by the seeds did not depend on the size (weight) of the embryo. Such a relationship was noted after 48 hours, and after 96 hours it was quite clearly visible (Table 11). At 7° there was no relationship of any kind observed between the size (weight) of the embryo and the amount of water absorbed by the entire seed. Such a situation is understandable since embryo activity appeared only after 168 hours.

The material presented shows that during the swelling process in maize seeds one can distinguish two phases: 1) Water absorbtion at the expense of forces exerted by the swelling of substances, and 2) water absorption at the expense of forces which arise as the result of embryo activity. The absorption of water during the first and the

TABLE 9. The Change in Water Content of Individual Parts of Maize Seed Depending on the Degree of Swelling at 7° (amount of water in one seed in mg and in % of the previous period)

Parts of seed	Hours after beginning of swelling											
	0	6		24		48		72		120		
Entire seed	29.73	50.8	171	98.1	193	113.0	115	135.4	120	139.2	103	
Endosperm	27.40	45.4	166	77.4	170	85.8	111	102.8	120	105.8	103	
Scutellum	2.00	4.3	215	18.4	428	24.3	132	29.1	120	28.0	96	
Embryo	0.33	1.1	334	2.3	209	2.9	126	3.5	121	5.4	154	

TABLE 10. The Course of Water Absorption by Individual Parts of Maize Seeds at 7° (in % absolute dry weight)

Parts of seed	Hours after beginning of swelling					
	0	6	24	48	72	120
Entire seed	12.7	18.7	41.6	45.6	57.4	59.2
Endosperm	12.9	20.5	37.6	39.2	50.0	50.2
Scutellum	10.4	18.8	70.7	91.0	121.1	101.3
Embryo	9.6	24.5	83.3	144.0	193.0	216.4

TABLE 11. Correlation Coefficient Between Dry Weight of the Embryo and the Amount of Water Absorbed by Maize Seed

Temperature, °C	Hours after beginning of swelling			
	24	48	96	120
22	−0.115	+0.466	+0.828	—
7	+0.353	−0.020	+0.107	−0.339

second phase depends in many ways on conditions. During the first phase it is determined by the structure of the substances stored in the endosperm and the hydration of the material, during the second phase absorption depends to a considerable degree upon conditions which affect the living parts of seeds, especially on temperature and the supply of air available to the seeds and embryo.

SUMMARY

Two phases can be distinguished in the absorption of water by maize seeds: 1) An initial phase of absorption which occurs as a result of the action of swelling forces; 2) absorption caused by forces due to the activity of living parts of the seeds when the latter begin to germinate. Absorption of water during the second phase is more dependent on environmental conditions than during the first.

At the beginning of swelling the water content first increases in the scutellum. Saturation of the endosperm sets in after 24 hours at a temperature of 22°, whereas the scutellum was saturated only after 48 hours. Subsequently, water mainly entered the embryo.

At a temperature of 7°, which is insufficient for germination of the seed, water mainly entered the endosperm, the uptake rate being almost the same as at 22°. Uptake of water by scutellum and embryo under these conditions was much slower. No movement of metabolites from the endosperm to the embryo occurred at 7° and this may be the reason that maize seeds do not germinate at this temperature.

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ON NICOTINE PRECURSORS

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Investigations of the biosynthesis of the products of plant vital activities are basically aimed at an understanding of the metabolic process during the entire period of plant growth and development. Among these products alkaloids are of great interest as specific metabolic substances formed by only certain plants. Therefore, the study of their formation and the elucidation of their role in the physiology of plants is a necessary part of our contemporary experimental program.

During the investigation of nicotine in relation to the growth and development of the tobacco plant it appeared to us that the synthesis of nicotine had already begun in the embryo and that nicotine moved into the seedling during very early stages in its development [1]. It has already been determined that carbon dioxide entering the roots of tobacco is utilized in the biosynthesis of nicotine [2].

It is quite possible to investigate the pathway and specificity of nicotine biosynthesis in tobacco plant roots by introducing into the plant along with the nutrients a suitable materials such as precursors or sources for particular parts of the nicotine molecule.

Published work on this problem can be divided into two groups. To the first group belong those studies concerned with the biosynthesis of nicotine in plants exposed in chambers containing $C^{14}O_2$. During photosynthesis under these conditions the plants form nicotine containing labeled carbon dioxide, a definite indication that nicotine is created during the normal plant growth process through plant assimilation of atmospheric carbon dioxide [3, 4]. To the second group can be relegated studies based on the introduction of some substance or other containing C^{14} or N^{15} into plant roots along with the nutrient solution. These studies are more numerous and comprehensive since by this method it is possible to introduce and then to trace a given component into the make-up of the nicotine molecule. These studies have shown that methionine, choline, betaine, glycine, formic acid, and formaldehyde are sources of the N-methyl group in nicotine; ornithine and glutamic acid enter the pyrilodine ring of the nicotine molecule [5-13].

How the pyrilodine ring is biosynthesized is still an unresolved problem; data from the literature are controversial regarding the participation of nicotinic acid as a percursor in this process [14-15].

As a part of a study of the precursors of nicotine we conducted experiments endeavoring to elucidate the role of certain substances such as plant metabolites and also to establish the connection between nicotine biosynthesis and the nitrogen and carbohydrate metabolisms.

Experiments were carried out in water culture with plants of Nicotiana tabacum preliminarily grown in a Knops nutrients solution initially one-fourth and then one-half normal salt concentration. This was done because the transfer of tobacco seedlings directly into full Knops solution severely retarded the plants. The experimental plants used were 2-3 months old, with well-developed root systems. Each plant was placed in a 500 ml vessel filled with Knops solution containing 100 μC of radioactive material added in two portions spaced five days apart. The plants were exposed for 11-13 days. After the designated time the plants were analyzed. The fresh plants were chopped and ground in a mortar. The nicotine was distilled with steam and the amount determined as nicotine dipicrate according to Pfilya [16]. The radioactivity of the dipicrate was measured with an end-window counter using a thick mica window of 1.9 mg/cm^2.

Analytical results from the two experiments are presented in Tables 1 and 2, respectively.

It appears that after the experiment the tobacco plants differed both on the basis of weight and their nicotine content. Apparently this also affects the specific radioactivity of both the plants themselves and the nicotine manufactured in them. It was found that the radioactivity of the nicotine was related to the form of carbon compounds from which the radioactivity was obtained.

Earlier experiments with $NaHC^{14}O_3$ showed that the formation of radionicotine occurred directly in the root system of the plants. If carbon dioxide fixation occurs in the roots as a result of its incorporation through a sequential cycle of biochemical conversions, then the extent to which this product of fixation is used is determined by the nature of the given compound and its linkage with other metabolic substances.

Since nicotine is a material of secondary origin, its path of synthesis in plants must, to an appreciable extent, depend upon the presence of compounds structurally similar to the components of the nicotine molecule or be a suitable radical.

We have conducted two experiments in which we used certain precursors which have already been established as important participants in the biosynthesis of nicotine [17].

Glycine -1-C^{14} and -2-C^{14} were selected to elucidate the incorporation of carbon from these compounds into nicotine in relation to the position of the carbon in

TABLE 1. Nicotine and Plant Radioactivity

Labeled Compounds Used C^{14}	Plant Dry Wt, g	Nicotine, mg	Radioactivity of Top Storey Leaves, cpm/mg	Radioactivity of Nicotine, cpm/min/ mM x 10^4
$NaHC^{14}O_3$	18.6	5.0	388	1.0
	23.4	10.8	370	0.7
$CH_2NH_2C^{14}OOH$	25.5	14.5	92	0.9
	21.9	18.1	248	0.8
$C^{14}H_2NH_2COOH$	11.8	11.8	38	12.5
	11.7	6.4	190	18.1
$C^{14}H_3COOH$	16.8	12.1	523	9.1
	18.0	7.9	480	10.8
$NaHC^{14}O_2$	13.9	9.9	168	2.7
	24.2	12.8	145	4.8

TABLE 2. Nicotine and Plant Radioactivity

Labeled Compounds Used C^{14}	Plant Dry Wt, g	Nicotine, mg	Radioactivity of Top Storey Leaves, cpm/mg	Radioactivity of Nicotine, cpm/min/ mM x 10^4
$NaHC^{14}O_3$	33.4	17.8	142	1.2
	49.5	22.3	96	0.7
$NH_2C^{14}H_3$	30.2	15.1	593	17.2
	25.4	10.7	680	15.0
$HC^{14}OH$	13.6	3.7	109	21.1
	15.9	7.7	109	9.0
$C_6{}^{14}H_{12}O_6$	15.9	6.1	190	6.0
	21.0	7.3	120	6.1
$C^{14}H_3COOH$	34.0	15.9	121	6.5
	23.0	11.2	163	7.5

the molecule of glycine. Acetic -2-C^{14} acid was employed along with glycine because we had shown earlier that the carbon from it was incorporated into nicotine and it was of great interest to compare it as an active metabolite. Formic acid was used as a precursor in the biosynthesis of the methyl group in nicotine. We set up the scheme of the first experiment with tobacco plants as follows (Table 1): 1) Sodium bicarbonate -C^{14}; 2) glycine-1-C^{14}; 3) glycine-2-C^{14}; 4) acetic 2-C^{14} acid; 5) formic acid.

The radioactivity of nicotine was the same in plants offered sodium bicarbonate-C^{14} and glycine-C^{14}. Apparently glycine-1-C^{14} undergoes decarboxylation and the carbon of the carboxy group as well as the bicarbonate carbon enter the nicotine molecule to a similar degree.

A completely different picture is observed with the incorporation of glycine-2-C^{14}. The radioactivity of nicotine appears to be 20 times greater than the radioactivity of nicotine in the preceding plants which contained bicarbonate-C^{14} and glycine-1-C^{14}. Consequently, methyl labeled glycine appears to be a much more active participant in metabolic reactions leading to nicotine synthesis. This view is supported by the fact that there is more radioactivity in the nicotine where acetic-2-C^{14} acid was used as a source than in the nicotine of the tobacco plants mentioned earlier.

Formic-C^{14} acid likewise causes the nicotine to be appreciably more radioactive than does bicarbonate-C^{14} and glycine-1-C^{14} indicating that is comparatively more available and active in nicotine biosynthesis reaction.

In the second experiment (Table 2) we endeavored to compare methylamine and formaldehyde as nicotine precursors. These substances are of great interest as metabolites since they may be direct sources of methyl groups entering the pyrolidine ring of nicotine.

Determinations show that when methylamine-C^{14} enters the plant the radioactivity of nicotine is 20 times greater than nicotine radioactivity when bicarbonate-C^{14} is used. Apparently, formaldehyde-C^{14} is similar to it in activity. Unfortunately, a mishap in the parallel experiment causing a blackening of the plant root system in one of the vessels forced a repetition of this experiment.

Most interesting in this experiment is the comparative introduction of labeled carbon into the plant as glucose-C^{14} and acetic-2-C^{14} acid. It appears that the radioactivity of the nicotine obtained from these plants was similar and almost seven times greater than the radioactivity of nicotine from plants growing in a solution containing bicarbonate-C^{14}. These data indicate that nicotine biosynthesis depends upon the carbohydrate (and nitrogen) metabolisms in that source materials for nicotine synthesis can be quite simple compounds containing

one or two carbon atoms but which appear to be active compounds in the metabolic process, as for example, acetic acid.

The plants utilized about 90% of the labeled compounds in solution. Incorporation of the C^{14} itself into nicotine was very slight, for example only 1% of the C^{14} in methylamine, 0.02-0.05% in the formaldehyde and 0.007% in the glucose.

Because we employed the isotope method, we had the opportunity to assess the part played by certain compounds in nicotine biosynthesis and to also distinguish their metabolic activity in relation to the position of C^{14} in the molecule of the compound employed.

These data show that nicotine is formed as a result of complex synthesis processes in which the carbon of bicarbonate, glycine, acetic acid, and also formaldehyde, formic acid, and methylamine may be involved. The incorporation of glucose carbon into nicotine serves as a direct argument for the hypothesis that nicotine synthesis is a result of carbohydrate and also nitrogen metabolism.

The theory that Robinson [18] has enunciated to the effect that amino acids participate in alkaloid formation finds support in work on the biosynthesis of nicotine using ornithine, lysine, glutamic acid, methionine and glycine as amino acid sources. The participation of glucose and acetic acid in nicotine biosynthesis indicates a link between nicotine biosynthesis and carbohydrate metabolism. The hypothesis that alkaloids are active products of metabolism which participate in one vital plant process or another is supported by the recent experimental work.

SUMMARY

Some experiments with radioactive precursors of nicotine were carried out in tobacco plants with the aim of studying the paths of alkaloid formation and elucidating the physiological role of these compounds in the plants. The following labeled compounds were introduced into a Knop solution: C^{14}-sodium bicarbonate, C^{14}-glycine, C^{14}-methylamine, C^{14}-formaldehyde, C^{14}-acetic acid, C^{14}-glucose; the activity was 100 μc per plant. The duration of the experiments was 11-13 days.

From the radioactive nicotine extracted from plants it could be concluded that C^{14}-methyl amine, glycine-2-C^{14}, acetic-2-C^{14}-acid and C^{14} glucose were the most active in biosynthesis of nicotine. It is suggested that biosynthesis of nicotine in plants is the result not only of nitrogen but also of carbohydrate metabolism.

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ON THE TWENTY-FOUR HOUR PHOTOSYNTHESIS OF PLANTS IN THE FAR NORTH

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The investigations of Kostychev, et al. [1] and Muller [2] showed that during the 24-hour polar day in the Far North photosynthesis in a number of arctic plants (Ivan-tea and polar birch) continues throughout the day and that its diurnal course is expressed by a unimodal curve with a maximum about "noon" and a minimum about "midnight."

This problem has not been subjected to much experimental study during the last twenty years.

In the work of Dadykin and Grigor'eva [3] which appeared in 1951 the authors agreed that, while photosynthesis in the wild birch (Betula pubescens) in the North (Igarka, 67.5°N) continues around the clock as long as the sun does not set, in cultivated plants like potato, assimilation was observed to approach a standstill for 4-5 hours at "night." The authors ascribed the differing reaction of these two species toward a long polar day to genetical differences between the two plants.

The findings of Dadykin and Grigor'eva [3] were confirmed by the work of Goncharik [4] who likewise observed the presence of a "nocturnal" interruption in potato photosynthesis in the North (Igarka).

Trulevich [5] observed that assimilation approached a standstill at Igarka during the "night" hours of a 24-hour day in other crop plants also (cabbage for 1.5 hr and sugar beets for 4.5 hr).

Analogous findings were also obtained in the experiments of Gorbunova [6] in central Yakutia (62°N): The wild plants, birch (Betula platyphylla) and (Rosa acicularis) assimilate around the clock; the crop plants, potato and maize interrupt assimilation during the "night time."

Even in the old papers of Kjellman [7] Fries [8], Smith [9] and Polunin [10] and the more recent papers of Pohjakallio [11-13] it was shown that crop plants introduced from the south and raised at northern latitudes accumulate a greater amount of organic material under long polar day conditions than during a shortened photoperiod just as native northern plants do.

In the work of Zhurbitskii and Vartapetyan [14] it was shown with the help of radiocarbon that, despite the fact that the assimilation rate in potato in the Far North (Kol'skii peninsula, 67.5°N) was 1.5 times greater during a 12-hour day than in the polar region, the total amount of carbon dioxide assimilated by the plants in a day was greater during a polar day then during a 12-hour day. From this it follows that a certain amount of carbon dioxide must also be fixed by the potato plants during the "night time."

In my 1956 paper (Kislyakova [15]) it was shown that over a four-hour period during a polar day (up to July 10) under conditions in the Far North (Kol'skii peninsula, 67.5°N) sprouts of Northern and Moscow potato varieties assimilated around-the-clock fixing at "midnight" 1.0-1.8 mg $CO_2/dm^2/hr$. I also showed that, depending on day length and light intensity during the "night time" (up to July 20), the nocturnal assimilation rate falls to 0.7-0.9 mg $CO_2/dm^2/hr$, but near August 1, when the day length decreased to 19.5 hours, potato photosynthesis was interrupted during the "night time."

Thus, the period of around-the-clock assimilation by the potato plant on the Kol'skii peninsula in the North is terminated by July 20-30.

The data we have obtained contradicts the data which have been presented from the literature and the question as to whether twenty-four-hour assimilation by crop plants in the North is possible requires detailed investigation. Therefore, in 1957 we repeated our experiments, expanded our objectives, and included plants which differ with respect to day length.

From among the cultivated plants we selected: soya (variety Kharbinskaya) as a typical short-day plant; Phaseolus (variety Gribovskaya) as a plant more or less day-neutral; potato (variety Early Rose northern strain) and grasses (local northern wheat Khibiny 314 and Lyutestsens 62 introduced from Moscow) as long-day plants. Blueberry (Vaccinium uliginosum) was selected from wild plants. The potato and grasses were grown in the field, the Phaseolus and soya were raised in a hothouse which was covered with a tarpaulin at night toward the beginning of July. During the remainder of the season the plants grew at ambient air temperatures. The blueberry plants were obtained from a nearby forest.

Photosynthesis experiments were carried out when the Phaseolus and soya plants possessed two true leaves, the potato was in the sprouting stage and the grasses were in the four-leaf stage.

As in the preceding work photosynthesis was determined by the method of Ivanov and Kossovich [16]. At the same time photosynthesis was determined we measured the light intensity (with a selenium light meter), cloudiness (visually), air temperature inside and outside

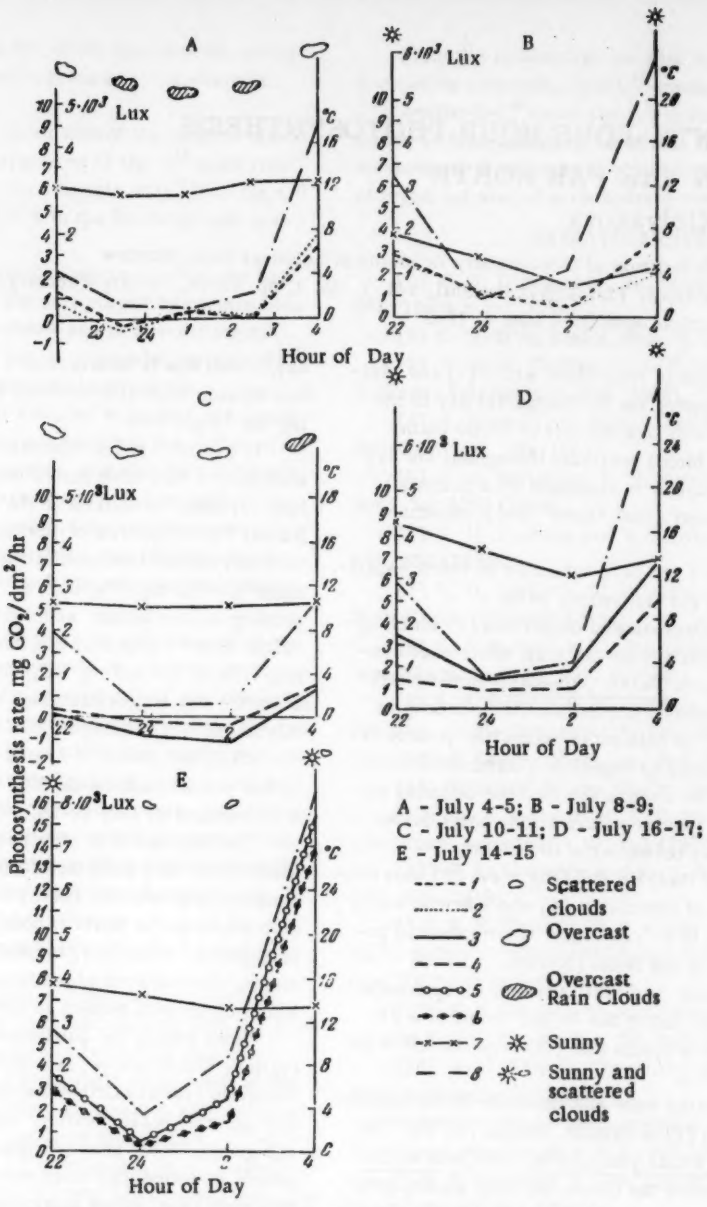

Figure. Rate of absorption and of carbon dioxide in different plants during the night (Apatity, 1957). A—July 4-5, B—July 8-9, C—July 10-11, D—July 16-17, E—July 14-15; 1) Soya; 2) Phaseolus, 3) potato northern strain, 4) blueberry, 5) Khibiny 314 wheat, 6) Lutestsens 62 wheat, 7) air temperature, 8) light intensity.

the flask and the condition of the stomata (Molish's method).

The experiments were carried out in 1957 on an experimental plot near the Apatity Station (67° 41'N) during a 23-24 hour day.

The results are presented in the figure.

Our data first of all fully support our 1956 conclusion that cultivated plants in the North possibly assimilate around the clock.

All of the crop plants investigated regardless of the photoperiodic group to which they belong can assimilate around the clock under the long-day conditions of the polar north (Figure, B, D, E).

Our 1957 experiment also revealed a close relation between "night" assimilation weather conditions, and light intensity.

During cloudy weather and with a low light intensity (250-300 lux) "night time" assimilation was either

absent or close to absent in both crop plants (Phaseolus, soya, potato) and in wild blueberry (Figure, A, C).

On the other hand, during clear "nights" with intense light (500-900 lux) both these plants and others assimilated successfully, the absolute amount of assimilate in Phaseolus and soya at "midnight" being 1.1-1.3 mg $CO_2/dm^2/hr$; in potato 1.4 mg, in wheat 0.4-0.45 mg, and in blueberry about 1 mg, that is, the amount of assimilates varied within the same limits as it did in our 1956 potato experiments (Kislyakova [15]).

The trend of assimilation rate during the "nocturnal" hours was determined chiefly by light intensity. Toward two o'clock in the "morning" with a certain increase in light intensity a slight increase was observed in the rate of assimilation. Only in the July 8-9 experiment (Figure, B) did the assimilation rate about 2 A.M. fall from 1.1-1.3 mg CO_2 to 0.59-0.66 mg $CO_2/dm^2/hr$. This effect was apparently linked with a prolonged fall in temperature at this time fell to 3.0°. About 4 A.M. the marked increase in light intensity was attended by a steep rise in the assimilation rate which in the July 14-15 experiment (Figure,E). reached 14.2 mg CO_2 in wheat variety Lyutestsens 62, 15.5 mg CO_2 in wheat variety Khibiniy 314, and in the July 16-17 experiment reached 6.9 mg CO_2 in potato and 4.9 mg $CO_2/dm^2/hr$ in blueberry.

Just as in the 1956 experiments plants introduced into the Far North meridionally have the ability to utilize nocturnal light just as do local plants. It is true, of course, that the northern wheat variety Khibiny 314 assimilated more rapidly during the night hours than the wheat variety Lyutestsens 62 introduced from more southerly regions. However, on the basis of our data it is difficult to say whether this is a special case or whether the northern wheat was more adaptive to the local conditions or had a better ability to use the nocturnal light for assimilation.

In conclusion, it is essential to point out that apparently the reason for the discrepancy between our results and certain data in the literature, should be sought either in the different climatic conditions of the region (Northern Kol'skii and Northern Asia) or in the different techniques employed about which we spoke in our first paper.

SUMMARY

Irrespective of the photoperiodic group to which they belong, not only wild plants such as blueberry but also cultivated plants (potato, cereals, bean plants) can photosynthesize twenty four hours a day under the long polar day conditions at the Far North (Kol'skii Peninsula, 67.5° N). The photosynthetic rate at night is mainly determined by illumination conditions. In cloudy weather, when the light intensity is low, apparent photosynthesis of cultivated as well as wild plants may be nil even over a twenty four hour polar day. The absolute photosynthetic rate in the night is low (around 1 mg $CO_2/dm^2/hr$). At four o'clock in the morning when the illumination begins to increase the photosynthetic rate also increases (3-15 mg $CO_2/dm^2/hr$).

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VARIATION IN NUCLEIC ACID AND VITAMIN CONTENT OF SOLANACEAE GRAFTS AND THEIR SEED PROGENY DURING ONTOGENESIS

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A number of studies have shown [1, 2, 3] that significant changes in metabolism occur as a result of grafting. Undoubtedly these changes will be reinforced with repeated graftings over a series of generations. It is of interest to study them not only in respect to phylogeny, but also in respect to the ontogeny of the plants in question.

It has been established [4-7] that nucleic acids and liponucleoprotein complexes play a substantial role in the most important vital processes of plants and animals. They participate in morphogenetic processes and in processes of protein synthesis.

Some of the causes of aging in organisms have been elucidated [8-10]. The investigators conclude that changes in synthesis and renewal of the protein molecule, in which certain liponucleoprotein complexes and particularly their component nucleic acids play an important role, are prominent among these causes. Alteration of these complexes affects protein renewal at various stages of ontogenesis.

In view of this it was our purpose to study the ontogenetic dynamics of nucleic acid and vitamin C accumulation in tomatoes and eggplant subjected to multiple grafts.

METHODS

Experiments were performed with grafted plants and with seed progeny obtained from multiple grafts of tomato and eggplant made over a number of generations. Grafted plants and seed progeny of the intervarietal combination Humbert tomato/Fikaratstsi tomato* and the interspecific combination Shtambovyi tomato/Grushevidnyi eggplant were used. Grafting was performed using cleft grafts; ungrafted plants of the varieties used in the grafts served as controls. In the series of experiments in which ontogenetic changes were studied, leaves were counted upwards along the stem. In the determination of nucleic acid and vitamin C content, leaves taken from the main stem, counted from the top, (fourth—see Table 2, and fifth—see Table 1) were analyzed.

An average sample consisted of 10-15 plants. Productivity (see Table 2) was evaluated with 25 plants and expressed on the basis of one plant. Samples were collected at 9:30 in the morning on a sunny day. Ascorbic acid (reduced form) was determined by the indophenol method of S. M. Prokoshev (see Petrochenko [11]). Ribonucleic acid, RNA, and desoxyribonucleic acid, DNA, were determined by the phosphorus method of Schmidt and Tannhauser, phosphorus by the Fiske-Subbarow method (see Belozerskii and Proskuryakov [12]).

RESULTS AND DISCUSSION

In Table 1 are presented results of studies† of the content of ascorbic acid, RNA, and DNA in leaves of graft hybrids of the combination Humbert tomato/Fikaratstsi tomato. A comparison of data for a fourfold graft and the seed progeny of a threefold graft reveals an increase of ascorbic acid content over the control, Humbert tomato, at all stages of development studied. Data in Table 1 for the graft of Shtambovyi tomato on Grushevidnyi eggplant indicate analogous changes.

It should be mentioned that in an earlier study with these same combinations [13] an increase in ascorbic acid content of leaves and fruits from single and double grafts was also noted. This increase may be explained as being due to the increased oxidation—reduction capacities of the hybrid plants which result from changes in metabolism.

If the accumulation of ascorbic acid is analyzed according to developmental stages (see Table 1), it is seen that the greatest quantity is accumulated in the flowering stage and that there is a decrease in the fruiting stage. This is in agreement with the data of Petrochenko [11], who studied ascorbic acid accumulation in potato leaves.

As Table 1 shows, there is also an increase in the nucleic acid content of grafted plants of both combinations and their progeny in the stages of bud formation, flowering, and fruiting. In view of the role of nucleic acids in protein synthesis and morphogenesis, it was of interest to compare data on variations in their content with plant productivity and rate of development.

The data of Table 2, and also those of other workers

*First term refers to the scion, second, to the stock; this system of designating a graft combination will be followed in the following discussion.

† V. L. Lityuk, a student, took part in some of the studies reported in Table 1.

TABLE 1. Changes in Content of Nucleic Acid and Ascorbic Acid in Leaves of the Graft Hybrids, Humbert Tomato /Fikaratstsi Tomato and Shtambovyi Tomato/ Grushevidnyi Eggplant, According to Development Stage (DNA and RNA in mg per g dry wt, ascorbic acid in mg % of dry wt)

Plant	Bud formation				Flowering				Fruiting			
	ascorbic acid	RNA	DNA	RNA/DNA	ascorbic acid	RNA	DNA	RNA/DNA	ascorbic acid	RNA	DNA	RNA/DNA
Fikaratstsi tomato (stock), control	573.5	2.22	0.90	0.40	606.9	1.12	1.06	0.94	270.4	0.96	0.32	0.33
Humbert tomato (scion), control	566.8	1.36	0.60	0.44	756.6	1.02	0.80	0.78	383	0.88	0.55	0.62
Humbert tomato/Fikaratstsi tomato, first seed generation of a threefold graft	621.4	1.77	1.20	0.67	788.6	0.86	1.55	1.80	480.5	0.89	0.95	1.06
Humbert tomato/Fikaratstsi tomato, fourfold graft	707.1	1.95	1.03	0.52	802	0.96	1.42	1.47	394	0.82	0.86	1.04
Grushevidnyi eggplant (stock), control	652.3	2.67	0.81	0.30	509.5	1.43	1.81	1.26	392.4	1.11	0.75	0.67
Shtambovyi tomato (scion), control	449.2	2.40	0.70	0.29	446.6	1.06	1.03	0.97	244	1.08	0.71	0.65
Shtambovyi tomato/Grushevidnyi eggplant, first seed generation of a twofold graft	485	2.59	0.97	0.37	485.5	1.01	1.51	1.49	251.2	1.38	0.99	0.71
Shtambovyi tomato/Grushevidnyi eggplant, threefold graft	503.7	3.17	1.77	0.55	557.9	1.55	2.08	1.34	271.1	1.21	0.80	0.66

[14, 15] show that changes in nucleic acid content of graft hybrids are correlated with the manifestation of hybrid vigor.

Turning again to Table 1, a certain parallelism between ascorbic acid content and DNA content may be seen; the maximum ascorbic acid content occurs at the beginning of the flowering period, at about the same time as the maximum DNA content occurs.

N. S. Turkova and L. A. Zhdanova [16] have shown that in a photoperiod favorable for the development of the variety in question the maximum DNA content is reached earlier than the maximum RNA content, and the DNA exceeds RNA in the flowering stage.

Our investigations are in agreement with the view of a number of workers [17] that possibly ascorbic acid is involved in nucleic acid conversions, especially in DNA formation.

The increases in ascorbic acid (reduced) and nucleic acid, especially DNA, which we observed in the flowering period provide a basis for the assumption that these changes are related to the transition from vegetative to reproductive growth. In order to test this assumpiton experiments were set up to study the relation between qualitative changes in the growing points during plant development and nucleic acid content of the leaves. Determinations were made of nucleic acid contained in leaves of the same age at different nodes along the main stem of Humberto tomato. Some of these data are presented in Table 3 and they show that the nucleic acid content of two-day-old leaves increased from the lower nodes up to the ninth or tenth node, i.e., the place at which the first inflorescence is forming; this increase is due to an increase in the DNA fraction. In leaves above the inflorescence, total nucleic acid content decreases as a result of a sharp drop in DNA, while there is a considerable rise in RNA content.

These data confirm those of previous experiments [13], which showed that the greatest increase in nucleoprotein of the terminal leaves of graft hybrids occurred at the stage of formation of floral buds; this, together with the enrichment of phosphorus in nucleoprotein, indicates not only a quantitative but also a qualitative change in this complex.

Some workers [16, 18] associate a change in nucleic acid metabolism of leaves and stems with the transition to reproductive development. The earlier flowering of graft hybrids which we have observed may be due, among other things, to

TABLE 2. Change in Nucleic Acid Content and Productivity of the Graft Hybrid, Humbert Tomato/Fikaratstsi Tomato

Plant	Total nucleic acid content of leaves at various stages (in mg P per g dry wt)			Wt. of green vegetative tissue, g	Length of main stem	No. of branches	No. of fruits	Wt. of fruit, kg	Wt. of largest fruit, g	No. of flowers
	Bud formation	Flowering	Fruiting							
Fikaratstsi tomato (stock), control	2.90	2.10	1.81	472	69	–	32	0.880	112	–
Humbert tomato, (scion), control	1.98	1.85	1.61	835	120	7	126	1.180	20	62
Humbert tomato/Fikaratstsi tomato, first seed generation of a single graft	2.32	2.11	1.75	1240	160	11	182	1.520	20	82
Humbert tomato/Fikaratstsi tomato, first seed generation of a fourfold graft	2.51	2.32	1.96	1800	130	13	262	2.155	20	102

a more vigorous metabolism in comparison with the controls.

One of the problems of our study was also to investigate the changes with age in nucleic acid content of the leaf, stem, and root of tomato and eggplant. A leaf from one node was analyzed at various ages, and also leaves of different ages from different nodes along the main stem. In the latter case samples were collected from different nodes of a single plant at one time.

Changes in nucleic acid content of the leaf from the second node of Shtambovyi tomato which occur with time were as follows (RNA and DNA in mg P per 100 g dry wt):

Leaf age (in days)	RNA	DNA
19	0.265	0.118
29	0.261	0.108
39	0.206	0.082
59	0.204	0.074

The nucleic acid content of leaves from various nodes of an 80-day-old Humbert tomato plant in which the first inflorescence was bearing fruit was as follows (nucleic acids in mg P per 100 g dry wt):

Leaf of node	Total nucleic acid
17	0.454
10	0.273
5	0.219

From these data it is possible to conclude that with leaf age the nucleic acid content steadily falls; this is explained by the reduced synthetic capacity of the tissues, which is characteristic of both plants and animals during aging [19, 20].

SUMMARY

1) The ascorbic acid content of leaves from the grafted plants and their seed progeny which were studied is higher than that of the control plants.

2) Ascorbic acid content of leaves is highest at the beginning of the flowering period, and during the fruiting stage it declines.

3) Nucleic acid content (RNA and DNA) of leaves of grafted plants and their seed progeny was in all cases higher than that of control plants.

4) In leaves of graft hybrids, and also in control plants, a certain parallelism in the accumulation of ascorbic acid and of DNA is evident. It is most vividly expressed in the period from bud formation to flowering. With an increase in the level of vitamin C, the DNA/RNA ratio is increased. This indicates that probably vitamin C plays a role in interconversions of nucleic acids.

5) It was established that nucleic acid content of leaves of Humbert and Shtambovyi tomatoes declines steadily with age.

6) The transition of tomatoes and eggplant from vegetative to reproductive growth is accompanied by metabolic changes which result in an increased accumulation of reduced vitamin C and nucleic acid.

7) Hybrid vigor, which is manifested in graft hybrids by morphological changes and by increased productivity, is associated with an increased nucleic acid content.

TABLE 3. Change in Nucleic Acid Content of Leaves of the Same Age (Two Days) with Nodal Position on the Main Stem of Humbert Tomato (phosphorus of nucleic acids in mg per 100 g dry wt)

Total plant age (in days)	Stage of development and No. of leaves on the main stem	Node from which two-day-old leaf came	Total nucleic acid	DNA	RNA
12	1-2	1-2	0.430	0.212	0.218
24	4	4	0.440	–	–
52	9-10•	9-10	0.470	0.274	0.196
152	25-26••	25-26	0.426	0.132	0.294

• Budding of first inflorescence.

•• Fruiting stage

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INTERACTION OF URACIL, RIBOFLAVIN, AND MALEIC HYDRAZIDE IN PLANTS

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Maleic hydrazide (MH) occupies a special place among compounds which inhibit plant growth and development. Plants which have been treated with MH solutions at certain concentrations remain for a lengthy period in the condition prevailing at the time of treatment, and neither develop further nor die. It is as if the plant were in a condition of narcosis [1]. The mechanism of action of this compound has long been obscure, although a number of opinions on this matter, which are frequently contradictory, have been published. Some workers [2, 3] believe that MH is an antagonist of indole acetic acid (IAA) and that it inhibits IAA synthesis in the plant. Audus and Thresh [4] and also Kulescha [5] suggest that this is not the case, but that MH increases indole acetic acid oxidase activity and thereby reduces the level of IAA in the plant. Muir and Hansch [6] have ventured the hypothesis that MH combines with sulfhydryl groups. This point of view should now be regarded as invalid in view of the recently demonstrated oxypyridase configuration of the MH molecule [7].

According to data of Isenberg, et al. [8], MH inhibits dehydrogeneses. Brian and Hemming [9] state that it possesses anti-gibberellin activity. One worker [12] has advanced the hypothesis that it acts as an antimetabolite to uracil in nucleic acid synthesis, basing his suggestion on the close similarity of MH and uracil (Fig. 1), on the studies of Ber [10] with thiouracil, an analog and antagonist of uracil, and on the absence of physiological activity in the simplest MH derivatives.

Similar suggestions have been made by Greulach [13]. In his experiments on the effect on germinating peas of MH in combination with compounds of the purine and pyrimidine series as well as certain other compounds, uracil, thymine, and thiouracil brought about a partial reduction of the activity of MH.

A number of cytological studies [14, 15], in which it was shown that MH causes an inhibition of mitosis in plants, should also be mentioned.

In our investigation we attempted to select those compounds which, when used in combination with MH, would reduce its inhibitory effect. Principal attention was directed to uracil, and also to riboflavin, which is able to catalyze photooxidations and thereby to reduce the inhibiting effect of high concentrations of such physiologically active compounds as IAA [16-18]. In certain cases we also tested other compounds, such as thymine, uric acid, alloxan, etc.

The effect of MH and other compounds was tested on young Gribov tomato plants and on germinating seeds of Muron cucumber.

Experiments with tomatoes were performed in the greenhouse or in a growing shed. Plants 8-12 cm high were thoroughly sprinkled with solutions of the given compound. Control plants were sprinkled with water. Where two compounds were used in combination, plants were first sprinkled with a solution of MH and then, after 1-2 hours, with a solution of the second compound.

Dry cucumber seeds were soaked 16-18 hours in the test solutions, then laid out on filter paper in Petri dishes which were kept in a controlled-temperature room at 22° for seven-eight days, either in the light or in the dark; at the end of this period biometric measurements and certain biochemical determinations were made. Control seeds were soaked in water.

In all experiments MH was used in the form of its diethanolamine salt at a concentration of 0.01 M. As preliminary tests indicated, this concentration was such as to inhibit growth and development sufficiently, but to permit resumption of growth after a certain interval of time.

In choosing uracil concentrations to be used, we decided on those high enough to inhibit plant development to a certain extent on the assumption that, in order to obtain any antagonistic effect with respect to MH, it would be necessary to use concentrations higher than those which are stimulating. On the basis of preliminary tests we chose a 0.05 M concentration of uracil. Riboflavin was used at a concentration of 0.0005 M.

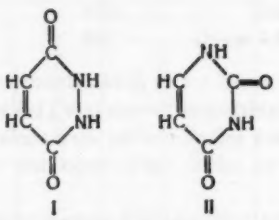
Results of Experiments with Tomato Plants

In Experiments with tomatoes, not only plant growth but also flower and fruit formation was considered. In Table 1 are data on the effect of treatment with MH and other compounds.

As these data show, in certain experiments uracil suppressed plant growth somewhat, while riboflavin had almost no effect in this respect. Treatment with MH led to a marked growth suppression (40-90%). In experiments performed in the summer, when physiological processes are most vigorous, MH was less inhibitory than

TABLE 1. Effect of Solutions of MH (0.01 M), Uracil (0.05 M), and Riboflavin (0.0005), Separately and in Combination, on the Growth of Tomato Plants (av of 12-15 plants)

Initial and final dates of individual experiments	Plant height at end of expt., in cm						The same in % of control				
	control	MH	uracil	MH + uracil	riboflavin	MH + riboflavin	MH	uracil	MH + uracil	riboflavin	MH + riboflavin
2/25-3/27	29	12	–	–	–	–	43	–	–	–	–
3/6 -4/26	19	10	–	–	–	–	52	–	–	–	–
4/16-6/17	66	40	66	55	–	–	60	100	83	–	–
5/24-6/30	77	41	66	61	–	–	53	85	79	–	–
10/13-11/17	52	11	55	16	53	17	21	105	30	100	31
10/18-12/22	116	10	104	12	112	12	9	89	10	96	10
The same including measurements of lateral branches	144	23	133	86	135	76	16	92	59	93	53



I II

Fig. 1. Structural formulas of maleic hydrazide (I) and uracil (II).

in the fall and winter. Evidently detoxidation processes are more vigorous in the spring and summer. When plants were treated with MH in combination with uracil or riboflavin, there was a partial removal of its inhibiting influence. In this connection it is interesting that while, in all treatments involving MH, the growth of the main stem was almost completely suppressed, that of the lateral branches was affected differently. Thus, data for the last experiment show that while the length of two lateral branches of plants treated with MH was 13.0 cm, in plants treated with uracil plus MH and riboflavin plus MH, the lateral branches were 74 cm and 64 cm long, respectively. In a count of growing points which had suffered injury, it was shown that all shoot apexes of plants treated with MH were damaged, while in plants treated with MH plus uracil or riboflavin 45-60% remained viable.

Plants treated with MH lagged behind control plants with respect to times at which flowering and fruiting took place and also with respect to amount of fruit formed. Treatment with uracil and riboflavin at the given concentrations had only a slight effect on fruit production. Treatment with uracil or riboflavin in combination with MH partially restored the fruit-bearing capacity (Tables 2 and 3).

Fig. 2. Tomato plants treated with MH, uracil, and a mixture of the two. 1—control; 2—MH; 3—MH + uracil; 4—uracil.

In conclusion of this section we present for illustration photographs of tomato plants of one experiment (Figs. 2 and 3).

Results of Experiments with Cucumber Seedlings

The experimental procedure of soaking cucumber seeds in solutions of MH, uracil, riboflavin, and a mixture of these compounds has been described above.

Results averaged from four experiments are presented in Table 4.

As these data show, soaking of seeds in a MH solution had a pronounced effect on growth; soaking in solutions of uracil or riboflavin did not affect growth ad-

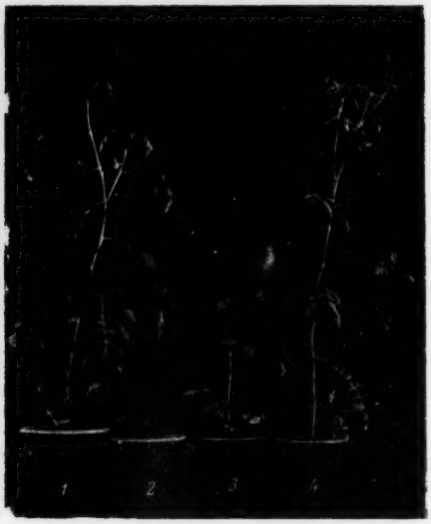


Fig. 3. Tomato plants treated with MH, riboflavin, and a mixture of the two. 1—control; 2—MH; 3—MH + riboflavin; 4—riboflavin.

versely. Addition of uracil to a MH solution reduced its inhibiting effect considerably, both when seeds were germinated in the light and in the dark. Addition of riboflavin was effective only in the light. Length of seedlings germinated in the dark after a soaking in solutions of MH + riboflavin was almost the same as that of seedlings after a soaking in a solution of MH alone.

Biochemical Changes Induced in Plants by Treatment With MH, Uracil, and Riboflavin, Separately and in Combination

The rate of respiration and the proportion of flavoprotein respiration in the shoot apexes of treated and control tomato plants were determined. Measurements were made in the Warburg apparatus. Potassium cyanide was used as a respiratory inhibitor (Table 5).

Results of the two experiments, which were performed at different times, showed the same pattern: Treatment with MH reduced the respiratory rate, and at the same time caused a considerable increase in the proportion of flavin respiration. Treatment with uracil had almost no effect on respiratory rate or on flavin respiration. Treatment with MH + uracil and MH + riboflavin tended to restore respiratory processes to normal: Respiratory rate and flavoprotein respiration values approximated those of control plants. At the same time, treatment with riboflavin alone resulted in a reduction of respiratory rate and in enhancement of flavoprotein respiration.

TABLE 2. Effect of MH and Uracil, Separately and in Combination, on Fruit Formation in Tomato Plants (av values per plant; single treatment at beginning of expt.)

Treatment	No. of buds	No. of flowers	No. of fruits	Total No. of fruit elements	% of control
Control	4.6	5.0	4.6	14.2	100
MH	0.0	0.0	0.0	0.05	0
Uracil	2.0	7.7	3.6	13.3	93
MH + uracil	2.8	2.8	0.9	6.5	46

With respect to cucumber seedlings, those from seeds soaked in MH suffered a reduction of respiratory rate, while those from seeds soaked in MH plus uracil showed an increased respiratory rate which was similar to that of control seedlings or seedlings treated with uracil alone, as shown by the following data (in μl O_2 per hr per g):

Compounds	Expt. 1	Expt. 2
Control	465	397
MH	292	272
Uracil	404	352
MH + uracil	388	303

These results are in good agreement with those obtained by Rakitin and Svarinskaya [19] with potato leaves treated with a solution of the diethanolamine salt of MH. Here, after an initial rise in respiratory rate, a decline also set in.

The riboflavin and IAA content of seven-day-old cucumber seedlings from seeds which had been soaked in solutions of MH, uracil, or riboflavin, or in mixtures of these compounds, was determined. Both determinations were made by fluorometric methods [20, 21]. Data obtained are summarized in Table 6.

As these data show, the riboflavin content of seedlings was reduced after treatment with MH or a mixture of MH and uracil; uracil alone also brought about a reduction, though to a lesser extent. When seeds were soaked in riboflavin or riboflavin plus MH, the riboflavin content of seven-day-old seedlings from these seeds remained high, being, however, somewhat lower in the case in which MH had been present. This provides a basis for the supposition that here there is a more vigorous riboflavin utilization.

With respect to IAA, MH and riboflavin reduced, and uracil markedly increased its content in seedlings. When MH was applied in combination with uracil or with riboflavin, there was a pronounced rise in IAA content. In this connection it is interesting that the increase was greater with MH + uracil than with MH + riboflavin.

These studies demonstrate that treatment with MH is reflected to a considerable degree in metabolic reactions, in reduced respiratory rate, altered respiratory patterns, and reduced riboflavin biosynthesis and IAA content. To a greater or less extent, addition of uracil and riboflavin restores the metabolism of MH-treated plants to normal.

TABLE 3. Formation of Fruit Elements in Tomato Plants Treated with MH, Uracil, and Riboflavin, Separately and in Combination

Initial and final dates of individual experiments	No. of fruit elements (av per plant)						The same in % of control				
	control	MH	uracil	MH + uracil	riboflavin	MH + riboflavin	MH	uracil	MH + uracil	riboflavin	MH + riboflavin
4/16-6/17	16.6	8.4	14.7	10.3	–	–	50.6	88.6	62.1	–	–
5/24-6/30	14.2	0.0	13.3	6.5	–	–	0.0	93.6	52.8	–	–
10/13-11/17	17.0	0.0	20.0	2.0	19	3	0.0	123.5	12.3	112.3	17.6
10/18-12/22	19.2	0.0	17.8	9.0	22	6.6	0.0	92.7	46.9	114.5	34.4

TABLE 4. Growth of Cucumber Seedlings after the Seeds Had Been Soaked in Solutions of MH, Uracil, and Riboflavin, Separately and in Combination (av of four experiments)

Compound	Concentration, M	In the dark		In the light	
		length, cm	in % of control	length, cm	in % of control
Control	–	10.0	100	8.4	100
MH	0.01	3.8	38	2.7	32
Uracil	0.05	9.5	95	8.3	98
MH + uracil	0.01 + 0.05	8.2	82	7.0	83
Riboflavin	0.0005	9.7	97	8.1	96
MH + riboflavin	0.01 + 0.0005	4.3	43	7.8	92

TABLE 5. Respiratory Rate and Proportion of Flavoprotein Respiration in Shoot Apexes of Tomato Plants Treated with Solutions of MH, Uracil, and Riboflavin, Separately and in Combination (data from two expts.)

Compound	Respiratory rate in μl O_2 per hr per g		% of flavoprotein respiration	
Control	387	473	27.5	40.5
MH	156	304	80.4	70.6
Uracil	328	456	30.5	40.1
MH + uracil	239	460	37.3	52.6
Riboflavin	–	300	–	76.3
MH + riboflavin	–	488	–	45.4

Participation of the Pyrimidine Ring of Riboflavin in Removal of the Inhibitory Effect of Maleic Hydrazide

The presence of a pyrimidine ring in the riboflavin molecule led us to make comparative tests of certain

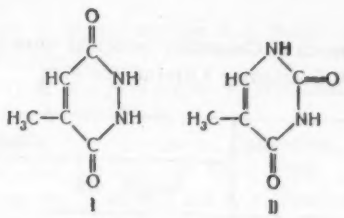


Fig. 4. Structural formulas of citraconic hydrazide (I) and of thymine (II).

compounds containing this ring, such as thymine, uric acid, and alloxan. In addition, citraconic hydrazide (CH), which has previously been synthesized [11], and which bears the same analogous relationship to thymine that MH bears to uracil (Fig. 4), was tested. Maleic hydrazide was tested at a concentration of 0.01 M, and citraconic hydrazide at a concentration of 0.03 M. All other compounds were tested at 0.0015 M, i.e., in concentrations three times greater than that shown to be active for riboflavin and 30 times smaller than that previously used for uracil. Results are summarized in Table 7.

These data show that at the concentrations employed only riboflavin was effective in counteracting the effect of MH. All other compounds, including uracil, failed to remove the inhibiting effect of MH. Citraconic hydrazide inhibited seedling growth less than did MH. Seedlings treated with 0.03 M CH suffered a certain retardation of growth, but to a smaller extent than with 0.01 M MH. Addition of thymine to the CH solution completely restored seedling growth. At a concentration of 0.01 M, CH had no effect on seedling growth.

Results of these experiments provide a basis for the assumption that uracil and thymine act as direct antagonists of MH and CH respectively, reducing their effect at equimolar concentrations (CH + thymine) or at greater concentrations (MH + uracil).

Thus, the hypothesis that uracil and MH are antimetabolites [12] has been experimentally verified.

TABLE 6. Riboflavin and IAA Content of Seven-Day-Old Cucumber Seedlings from Seeds Which Had Been Soaked in Solutions,of MH, Uracil, or Riboflavin, Separately or in Combination

Compound	Riboflavin (germination in light)		IAA; germination			
			in the dark		in the light	
	in μg per 10 seedlings	% of control	in μg per 10 seedlings	% of control	in μg per 10 seedlings	% of control
Control	3.66	100	2.3	100	2.64	100
MH	2.92	79	2.0	86	1.66	62
Uracil	3.25	88	2.5	108	3.90	147
MH + uracil	2.66	72	6.0	260	6.60	250
Riboflavin	8.69	237	–	–	1.85	70
MH + riboflavin	7.33	200	–	–	3.48	131

TABLE 7. Growth of Cucumber Seedlings from Seeds Which Had Been Soaked in Solutions of MH, CH, and Other Compounds Containing a Pyrimidine Ring

Compound	In the dark		In the light	
	length, cm	% of control	length, cm	% of control
Control	12.4	100	9.1	100
MH	5.4	44	4.1	51
Uracil	11.7	94	10.2	112
MH + uracil	5.8	47	4.5	50
Riboflavin	12.3	100	9.4	103
MH + riboflavin	6.3	51	9.6	105
MH + thymine	6.3	51	4.5	50
MH + uric acid	6.4	52	4.5	50
MH + alloxan	5.2	42	4.2	46
CH	6.9	56	5.7	64
CH + thymine	8.7	70	6.9	76

NOTE: Concentrations employed, MH—0.01 M; CH and CH + thymine—0.03 M; all other solutions—0.0015 M.

Riboflavin modifies the inhibiting effect of MH in some other, more complex fashion. This follows from the fact that not one of the compounds with a pyrimidine ring which was tested reduced the effect of MH in the small concentrations at which riboflavin is effective. A comparison of effective concentrations of MH, riboflavin, and uracil reveals that the concentration of riboflavin is 20 times lower than that of MH, and 100 times lower than that of uracil, and on the basis of the pyrimidine ring it is even 300 times lower. Moreover, riboflavin is active only in the light. This compelled the assumption that here the sensitizing property of riboflavin was evidently playing a basic role. According to investigations of Kolesnikov [22], riboflavin is able to oxidize phenols in the light. Maleic hydrazide may also have a structure similar to that of phenols. Moreover, Andreae [23] showed by in vitro experiments that riboflavin catalyzes the oxidation of MH. This led us to perform direct experiments on the oxidation of MH by riboflavin in the light. They were carried out in a Warburg apparatus modified for this purpose by P. A. Kolesnikov, to whom we extend our thanks for his cooperation in making these tests.

The absorption of oxygen by MH in the light in the presence of riboflavin is shown by the following data:

Composition of mixture	Concentration (in mg per 5 ml)	O_2 absorption (in μl per hr)
MH	3	4.4
Riboflavin	0.075	6.8
MH + riboflavin	3 + 0.075	25.8
Riboflavin	0.15	7.3
MH + riboflavin	3 + 0.15	33.0

As in the in vitro experiments, there was a slight but definite oxidation of MH in the light in the presence of riboflavin. At the termination of the experiment it was possible to detect traces of hydrogen peroxide formed in the reaction. Possibly oxidation of MH by riboflavin is more vigorous in plants in the presence of various enzyme systems.

SUMMARY

Maleic hydrazide (MH) is an antimetabolite of uracil which counteracts the inhibiting effect of MH on growth and development of plant. It is possible that MH inhibits the biosynthesis of ribonucleic acid.

Riboflavin removes the inhibiting effect of MH as a result of its sensitizing properties, probably by catalyzing the oxidation of MH in light.

The decrease in the amount of indole acetic acid which MH causes, and also the pronounced increase in indole acetic acid content which is observed when uracil or riboflavin are introduced together with MH indicates that this acid participates in the removal of the inhibiting effect of MH.

From the foregoing it can be concluded that the inhibiting action of MH can be counteracted in various ways with the participation of riboflavin, uracil and possibly indole acetic acid and other systems.

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BRIEF COMMUNICATIONS

BIOSYNTHESIS OF PHYTOL IN THE DARK BY ANGIOSPERMS

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In a previous study [1] it was shown that certain angiosperms are able to synthesize chlorophyll in the complete absence of light. In a series of experiments with carefully purified chlorophyll a and chlorophyll b preparations from Ceratophyllum, Elodea, Potamogeton, and bean plants which had been kept in darkness in the presence of $C^{14}O_2$ or $Na_2C^{14}O_3$, a fairly high C^{14} content was observed.

In this study, however, we left the question of C^{14} distribution in the chlorophyll molecule open. It was first necessary to determine whether C^{14} is incorporated equally into the phorbin and phytol moieties, or whether it is incorporated predominately, or even exclusively, into one moiety or the other.

We therefore set up experiments along the same lines with garden cress. In these experiments we set ourselves the task of obtaining, if possible, higher amounts of carefully purified chlorophyll a and b preparations so that they could then be measured for C^{14} content.

Experimental procedures employed were those described previously [1]. Dark exposure was for a period of four days.

Chlorophyll preparations, obtained by a method which ensures complete purity [1], were hydrolyzed with sodium hydroxide in methyl alcohol and separated by chromatography with a methanol–diethyl ether solvent system into chlorophyllin and phytol fractions, which were then analyzed for radioactivity. Repeated analyses were made in order to surmount the disadvantage of low reproducibility of results, which is due to difficulties inherent in the study of such preparations. Results obtained are presented in the table.

TABLE. Relative Radioactivity of Phytol Obtained from Chlorophyll a and Chlorophyll b Preparations

Expt. No.	Measured radioactivity, counts/min		Radioactivity of phytol, adjusted for self absorption, counts/min	Relative radioactivity of phytol (in % of chlorophyll radioactivity)
	chlorophyll	phytol		
			Chlorophyll a	
I	10.9	5.3	10.0	91
	11.5	3.7	7.0	61
II	17.2	6.6	16.3	95
III	20.2	7.2	16.1	80
	16.4	8.5	17.2	105
IV	26.1	12.2	25.7	99
V	22.4	9.8	19.7	88
Av (chlorophyll a)				89 ± 7
			Chlorophyll b	
I	4.0	1.4	2.6	65
	3.8	2.4	4.2	114
II	5.84	4.0	9.0	151
III	4.9	2.2	4.9	103
	6.25	3.8	8.3	124
IV	13.3	7.8	15.5	117
V	6.64	2.8	5.64	85
Av (chlorophyll b)				108 ± 13
Av (chlorophyll a and chlorophyll b)				99

As these data show, practically all the radioactivity was concentrated in the phytol portion of the molecule. We did, however, sometimes observe a very slight activity in the chlorophyllin portion as well. In these cases, though, hydrolysis had usually been accomplished with

potassium hydroxide, and the observed activity might have been due to a natural radioactivity of potassium. Thus, with garden cress we were able to establish with a fair degree of certainty the ability to synthesize phytol in the dark, although we could not demonstrate dark synthesis of the phorbin moiety. It is difficult to say at this time whether the absence of dark synthesis of the phorbin moiety is general for all angiosperms. Certain data indicate that Elodea is capable of such synthesis in very limited degree (as shown by experiments of Kutyurin [2] with heavy nitrogen and by experiments of Robbelen [3] on etiolated Arabidopsis plants using a spectrophotometric method), as are a number of other plants, according to studies of absorption spectra of their extracts which were made by Godnev and Akulovich [4].

In the future we hope to set up experiments on dark synthesis of chlorophyll, using other plants, in an attempt to obtain preparations of high activity with which we can establish more definitely whether dark synthesis of the phorbin moiety does in fact occur.

It has been shown by basic hydrolysis of C^{14}-labeled chlorophylls a and b extracted from garden cress which had been grown in the dark in a $C^{14}O_2$ atmosphere that practically all the radioactivity is localized in phytol. This indicates that there is a substantial synthesis of phytol in the dark. In order to decide whether dark synthesis of the phorbin moiety occurs, as some data indicates, further experiments are necessary.

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DEPENDENCE OF ONION, LETTUCE, AND CABBAGE YIELD ON THE NITROGEN, PHOSPHORUS, AND POTASSIUM CONTENT OF THEIR LEAVES

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Leaf diagnosis, i.e., a method of determining fertilizer requirements on the basis of chemical analysis of leaves, is based on a dependence of growth on leaf mineral content, which has as yet been little studied.

The underlying idea in attempts to use tissue analysis in yield prognosis is this: Only those mineral nutrients which are present in plant organs have an effect on growth rate and on yield [1]. For a complete and accurate diagnosis, therefore, the relationship between growth and content of one or another mineral element in the leaves should first be determined.

Study of this relationship is complicated by a number of circumstances. According to Steenbjerg [2], when soil mineral levels are adequate, leaf mineral content will gradually fall as yield increases. With introduction into the soil of a mineral element which had previously been at a minimum, its concentration in the leaf at first falls as yield increases. Only with an increase in dose applied, together with a further increase in yield, does the concentration of the given mineral in the leaf increase. Hence, as Steenbjerg emphasizes, ratios of individual elements are of no great assistance in a correct evaluation of results of chemical analysis of leaves. According to studies of Prevo and Ollan'e [3], such ratios are of great significance in relation to yield.

Determination of the content of a given element, for example nitrogen, which occurs in various compounds, is often of less value than determination of separate compounds. Thus, in diagnosis of the nitrogen requirement of sugar beet, it was more important, and easier, to determine nitrate content of leaf petioles than to determine other forms of nitrogen [4, 5]. Determination of phosphorus soluble in 2% acetic acid gave better results for diagnostic purposes than determination of total phosphorus [6, 7, 8].

The dependence of plant growth on soil type, soil levels of macro- and microelements, illumination, moisture, temperature, etc., is generally known. Nevertheless, a study of the dependence of growth on mineral content of leaves and other organs is of great promise. Such a study offers the possibility that in the future empirical experiments with fertilizers may be replaced by a rational use based on deeper understanding of plant nutrition.

We have attempted to study the dependence of growth on mineral nutrient concentrations in leaves of leafy vegetables. This relationship is more accessible to study than in plants whose economic value resides in tissues other than those of the leaves. The leaf yield of lettuce, onion, and cabbage coincides for all practical purposes with the accumulation of aerial tissues, i.e., with growth during the growing period.

Experiments with fertilizers were carried out according to the following scheme: O, K, PK, N/2PK, NPK. Plants used were head lettuce, onion, and two varieties of cabbage. Experiments were performed in 1955 and 1956 at L'vov, under conditions of adequate precipitation, on gray forest soils of different texture sandy loam and clayey loam.

Experiments with lettuce and onion were performed in 1956 at the university botanical garden on two-meter plots of clayey loam and sandy loam. The total nitrogen contents of the two soils were 0.194% and 0.132%, respectively, and the easily hydrolyzable nitrogen contents per 100 g were 6.83 and 5.15 mg, respectively. The experimental scheme employed and the yield obtained are shown in Table 1. The field portion of the experiment was carried out by E. A. Rubanyuk and A. A. Ermakova, colleagues in the Department of Plant Physiology.

Determinations of total nitrogen, phosphorus, and potassium were according to generally used methods [9]. Leaf protein nitrogen was determined according to Mobe [10].

The uptake of nitrogen, phosphorus, and potassium by lettuce and onion under various fertilizer regimes is shown in Fig. 1. A comparison of curves for yield and for mineral absorption permits determination of the role of the latter in crop production. The curves for potassium absorption and leaf yield are seen to be parallel, especially in onion. Curves for phosphorus absorption and yield show a smaller correlation. At high nitrogen doses, increase in phosphorus absorption by onion is less than increase in yield, and in lettuce is completely absent.

In our experiments application of potassium and phosphorus did not increase yield; only nitrogen fertilizer had this effect. With respect to nitrogen fertilizer, both lettuce and onion exhibited peculiarities in response. In

TABLE 1. Average Weight (in G) of the Aerial Portion of a Plant Supplied with Mineral Fertilizers (av of 40 plants)

Fertilizer, g/m²			Lettuce		Onion	
NH_4NO_3	superphosphate	KCl	clayey loam	sandy loam	clayey loam	sandy loam
–	–	–	32.2	41.2	27.7	12.5
–	–	10	35.2	41.0	27.4	12.6
–	20	10	58.2	35.0	25.2	13.9
10	20	10	68.0	76.2	27.0	19.1
20	20	10	72.3	93.4	33.0	24.2

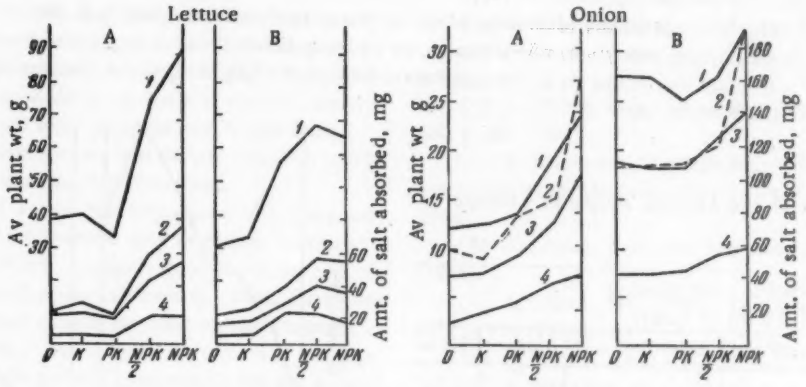

Fig. 1. Yield of lettuce and onion, and uptake from the soil of various mineral nutrients in relation to fertilizer regime. A—plants grown on sandy loam; B—on clayey loam; 1—wet wt.of plant; 2—absorption from the soil of nitrogen; 3—of K_2O; 4—of P_2O_5.

lettuce, yield increase was accompanied by a corresponding increase in nitrogen absorption, while in onion nitrogen absorption outstripped yield increase (Fig. 1), indicating that with high nitrogen doses new organic factors become important.

Thus, yield (growth) of onion and lettuce is a response mainly to nitrogen absorption, which is the fundamental factor determining yield of these crops. But in the course of nitrogen incorporation the effect of other conditions is either enhanced or suppressed, and new growth factors arise whose combined influence also determines final yield.

The dependence of average plant weight on leaf content of nitrogen, phosphorus, and potassium is shown in Fig. 2. Leaf phosphorus content in both lettuce and onion was practically constant and declined only slightly with nitrogen fertilizer. Potassium content also varied little, being slightly increased with nitrogen application. Nitrogen content of the leaves was the most variable. At low doses of nitrogen, it showed no increase. Only when the nitrogen dose was increased, together with a considerable increase in yield, did the leaf nitrogen content rise. Leaf analysis revealed the cause of the unexpected yield increase of lettuce on clayey loam to which PK fertilizer had been applied as opposed to the absence of such an increase on sandy loam. It turned out this was due to an increase of leaf nitrogen on soil of the former type.

High yields of lettuce and onion at high nitrogen dose levels were associated with an increase of the nitrogen:phosphorus ratio in the leaves. This increase was due not only to an increase in nitrogen percentage, but also to a decrease in phosphorus percentage.

Yield increase in onion was closely related to accumulation of nonprotein nitrogen, which is one of the signs of an abundance of nitrogen in plant tissues (with normal soil moisture levels, of course). The presence of nitrate nitrogen is also a sign of nitrogen abundance, which explains the value of determining nitrates as a criterion of adequate nitrogen supply [5]. Obviously, simple measurements of soluble nitrogen will serve as one of the diagnostic criteria of a nitrogen requirement by plants.

Experiments with cabbage yielded similar results. They were performed using two varieties of cabbage. Variety Slava was grown on sandy loam at the botanical garden of L'vov University, and variety Amager on sandy loam at the Zhdanov collective farm near L'vov. The content of easily hydrolyzable nitrogen (in mg per 100 g of soil) was 5.55 at the botanical garden and 5.37 at the farm. Planting times and agricultural techniques em-

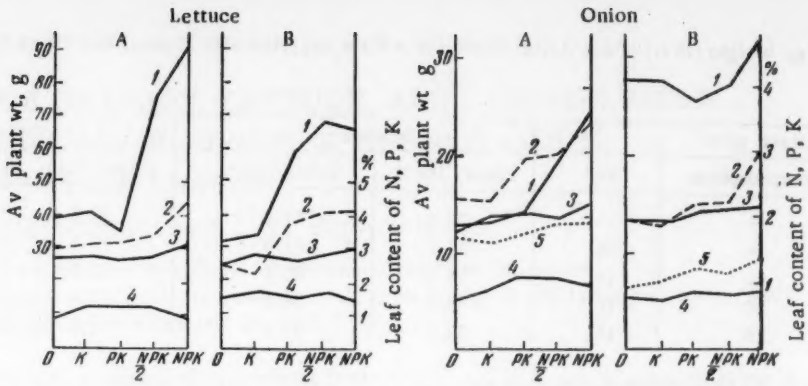

Fig. 2. Dependence of average plant weight on leaf content of nitrogen, phosphorus, and potassium. A—plants grown on sandy loam; B—on clayey loam; 1—plant wet wt.(in g); 2—total nitrogen; 3—K_2O; 4—P_2O_5; 5—protein nitrogen (in % of dry wt).

TABLE 2. Fertilizer and Average Weight of Cabbage Heads (in Kg)

Fertilizer applied to each plant, g (dissolved in 400 ml of water)				
NH_4NO_3	superphosphate	potassium salt	Amager	Slava
—	—	—	1.24	0.95
2	10	7	1.80	1.20
5	10	7	2.11	1.42
8	10	7	2.23	1.65
12	2	7	2.65	1.86
12	4	7	2.95	2.36
12	6	7	2.53	1.80

ployed were the same so that yield differences were due to varietal differences.

Fertilizer was applied in the form of solutions on July 1 and August 5. In experiments with variety Slava, there were 40 plants in each experimental group, and with variety Amager, 90 plants. Harvesting and yield count were performed at crop maturity.

The scheme of the experiment and yield data are presented in Table 2.

The dependence of cabbage yield on nitrogen and phosphorus contents of the outer leaves at different times is shown in Fig. 3. Application of increasingly large nitrogen doses led to a pronounced increase in yield and was associated with the accumulation of nitrogen in the leaves (after minimal requirements had been satisfied).

The highest yield was obtained at a ratio of ammonium nitrate to superphosphate equal to three. Under this fertilizer regime, however, there was no substantial change in the nitrogen:phosphorus ratio of the outer leaves, this differing but little from that at the less effective fertilizer ratios of 6 and 2. Obviously, the maximal effectiveness of the optimal fertilizer regime is due

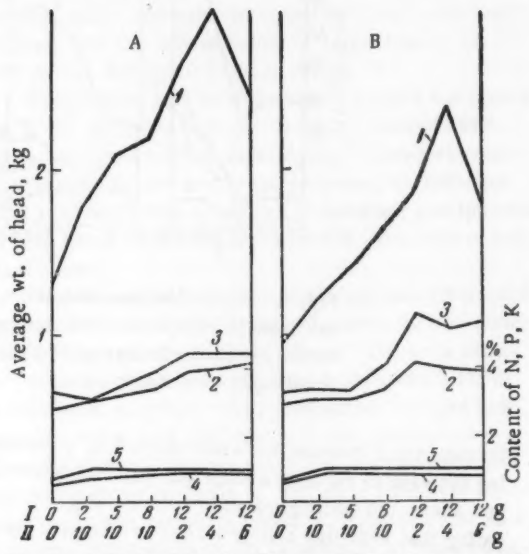

Fig. 3. Relationship of cabbage yield and content of nitrogen and phosphorus in the outer leaves at different times. A—var. Amager; B—var. Slava; 1—wet wt.of a head (in kg); 2—total nitrogen on Aug. 5; 3—the same on Sept. 1; 4—P_2O_5 on Aug. 5; 5—the same on Sept. 1; I—ammonium nitrate doses; II—superphosphate doses.

not to mineral ratios in the leaves, but to the influence of some additional growth factors, perhaps to an enhancement of the effect of phytohormones [1].

The fact that initial increase in yield after nitrogen application is associated not with an increase in leaf nitrogen content, but with a reduction in the nitrogen:phosphorus ratio, confirms the conclusion of Steenbjerg [2]. This conclusion, however, is applicable for comparatively low yields of leafy vegetables. In our experiments, maximal yield was in all cases accompanied by a marked increase in the nitrogen:phosphorus ratio (see Figs. 2 and 3).

Thus, determination of the nitrogen:phosphorus ratio of leaves acquires diagnostic significance only at high nitrogen levels.

The question arises: What nitrogen content can be considered sufficiently high? This question is a complicated one. The optimal content is not the same for all soils. In onion, a smaller nitrogen content was associated with a higher yield on clayey loam than was the case on sandy loam, and in lettuce, at a given leaf nitrogen content, yield on sandy loam was higher than on clayey loam (Fig. 2). In the less productive Slava cabbage the leaf nitrogen content was higher than in the more productive Amager cabbage.

From this it follows that optimal nitrogen content and optimal ratios of nitrogen to other elements should be individually determined for each variety and for given soil-climatic conditions. In our experiments, analyses of potassium and total phosphorus of leaves turned out to be inadequate for purposes of leaf diagnosis unless accompanied by nitrogen determinations.

Figures 2 and 3 show that high yields of lettuce, onion, and cabbage are possible only when leaf nitrogen content is high, though relatively good yields may be obtained even at small nitrogen contents. Thus, a high onion yield is obtained only if the total nitrogen content of the leaves is about 3%, while for lettuce and cabbage it must be above 4% and 5%, respectively (of dry weight).

The general similarity of patterns for the three plants should be kept in mind, however, regardless of their suitability for practical purposes. Obviously, progress in this area will depend on a thorough study of the interactions of absorbed elements with internal and external growth factors. A possible approach to this problem would be a study of correlations of growth with certain compounds in the leaves which contain the mineral nutrient elements, in particular such compounds as proteins, amino acids, nucleic acids, etc.; such a study would at the same time promote understanding of their functional role.

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ON THE FORMATION AND CONVERSIONS OF ALKALOIDS DURING GRAFTING

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The formation and conversions of alkaloids in plants have long engaged the attention of investigators. Their role in the plant's economy is still unknown, however.

Pathways of alkaloid biosynthesis, especially that of nicotine, have not as yet been fully elucidated. A knowledge of biochemical processes leading to the formation of nicotine would be of great scientific interest.

It has been demonstrated by grafting procedures that the root system is the site of nicotine and atropine synthesis [1-7].

Studies of Il'in [4d] have shown that roots of N. tabacum can take up bicarbonates. Carbon dioxide originating from them can be utilized in synthesis of C^{14}-labeled nicotine only if the root system is present.

Studies of Kursanov [9, 10] on the role of the root system in the synthesis of amino acids and other compounds have played a prominent part in the demonstration of nicotine synthesis from amino acids by roots.

The roots, utilizing mineral nitrogen and carbon dioxide from the soil, are thus able to synthesize amino acids, and then by further conversions to synthesize alkaloids.

Experiments with sterile cultures of isolated roots which have been treated with nicotine acid labeled with H_2 and C^{14} have shown that nicotinic acid is the precursor of the pyridine ring [11]. The amino acid ornithine is the source of the pyrrolidine ring [12, 13]; C^{14}-glycine is incorporated into the methyl group of the pyrrolidine ring of nicotine [14].

It is postulated that alkaloids or the process of their formation is vitally important to plants [15-22].

In our experiments we grafted alkaloid-containing to alkaloid-free plants with the purpose of elucidating the problem of nicotine formation. In the graftings we employed makhorka tobacco (N. rustica), an alkaloid-containing plant, and Bizon tomato (Solanum lycopersicum), Berlkhengen potato (S. tuberosum), black solanum (S. nigra var. vulgare), and fragrant tobacco (N. affinis), all alkaloid-free plants. Experiments were performed from 1950 to 1956.

Plants with six-seven leaves were grafted using cleft grafts. Grafted plants were kept in a damp chamber 7-10 days, then in a warm room for five-six days, and finally they were planted outside. At the end of the growing period they were dried in a drying oven at 50-60°. Nicotine was removed by steam distillation, precipitated with picric acid, and determined according to the method of Pfilya and Schmidt [23].

Investigations showed that when makhorka tobacco is grafted onto tomato, potato, solanum, or fragrant tobacco, it loses the ability to synthesize its native alkaloid, nicotine, in a year. Nicotine synthesis does not occur even when only the root system is lacking (experiment of 1954). It was also shown that tomato and potato scions synthesize alkaloids not characteristic of them when only the root system of makhorka is retained.

As examples of this type of experiment we shall present only data from the 1951 experiments with five potato plants grafted onto the root system of makhorka tobacco plants (grafting June 22), and data from the 1956 experiments with six tomato plants (grafting May 16). In the first case, the nicotine content of the potato scion (in % of dry weight) was as follows: 0.56; 0.68; 0.46; 0.29; 0.40—average 0.48%. In the second case (scion, tomato) the values were 0.30; 0.37; 1.60; 0.93; 1.10; 0.48—average about 0.80%. Control makhorka tobacco plants in 1951 contained 1.20% nicotine, and in 1956, 1.10%.

In 1955-1956 we rooted cuttings from a tomato scion containing nicotine (graft of tomato on makhorka tobacco) in distilled water. After rooting (5th-10th day), we planted the cuttings outside together with the grafted plants from which they had come. In the fall, at the end of the growing period, nicotine was determined in the tomato scions and in the plants obtained from cuttings. Analyses showed (Table 1) that the rooted cuttings had lost the ability to synthesize nicotine.

These results confirm those of A. A. Shmuk, G. S. Il'in, M. M. Tushnyakovaya, Dawson, and others showing that the root system is of crucial significance in nicotine synthesis.

The behavior of scions of fragrant tobacco (N. affinis) grafted onto makhorka tobacco is different.

According to results of experiments performed in 1950, nicotine is formed in fragrant tobacco only when the leaf surface of the stock predominates. If only the root system of the makhorka tobacco stock is present, nicotine is not synthesized. These results were verified the following years, and results obtained are presented in Table 2.

From Table 2 it may be concluded that if the stock possesses a well-developed foliage component, nicotine

TABLE 1. Nicotine Content of Tomato Scions (Grafts of Tomato on Makhorka Tobacco) and Cuttings from These Scions (in % of dry weight)

Nicotine content	
tomato scion	cutting from this scion
	1955 expt.
0.96	None
0.80	None
	1956 expt.
0.48	None
0.48	None
0.30	None
0.37	None
0.93	None
1.10	None
1.60	None

TABLE 2. Nicotine Content of Components of a Fragrant Tobacco/Makhorka Tobacco Graft in Relation to Foliage Development (Nicotine was determined according to the formation of the dipicrate after addition of picric acid to the steam distillate).

Leaf dry wt, g	Nicotine content*
scion/stock	scion/stock
1951 Expt., grafting June 22, analysis Sept. 15**	
17.75/10.22	Precipitate/abundant precipitate
4.48/ 3.35	Precipitate/precipitate
8.21/ 4.71	Precipitate/precipitate
5.21/ 2.48	Precipitate/precipitate
7.88/ 6.51	Precipitate/abundant precipitate
1951 Expt., grafting June 22, analyses Sept. 17***	
7.20/ 1.12	No precipitate/precipitate
13.1 / 1.24	No precipitate/precipitate
15.2 / 1.35	No precipitate/precipitate
12.5 / 1.55	No precipitate/precipitate
1953 Expt., grafting June 18, analyses Sept. 25****	
–	Precipitate/abundant precipitate
–	Precipitate/abundant precipitate
–	Precipitate/abundant precipitate
–	Precipitate/abundant precipitate
–	Precipitate/abundant precipitate
–	Precipitate/abundant precipitate

*In makhorka tobacco controls, abundant precipitate, and in fragrant tobacco controls, no precipitate.

**Leaf surface area of stock and scion approximately equal.

***Leaf surface area of fragrant tobacco scion greater.

****Leaf surface area of makhorka tobacco stock greater.

is formed in both scion and stock. It is true that in the scion this is significantly less than in the stock. We observed the same thing in 1950 in the graft combination, fragrant tobacco/makhorka tobacco, where nicotine content of the scion was 0.32% and that of the stock was 1.2%, while leaf area of the scion was 113.4 cm^2. Where leaf area of the scion exceeded that of the stock, however, no nicotine was formed in the scion. The occurrence of nicotine in the stock of these plants indicates, apparently, that it is transported into the scion but that it undergoes transformations there.

It is difficult to say just what transformations occur in the scion, but nevertheless it can be postulated that nicotine is converted into some other alkaloid. Examples of interconversions of alkaloids in leaves of tobacco (N. tabacum) grafted onto N. glauca have been noted by Il'in [4, 6]. Nicotine may, alternatively, be degraded, as in leaves of tobacco during autolysis and starvation [22, 29]. It is possible that it is enzymatically converted, as in ripening of N. tabacum seeds: Ripe seeds do not contain this alkaloid [24]. The validity of these hypotheses will be tested in further studies.

The following conclusions may be drawn from these experiments.

A fragrant tobacco (N. affinis) scion on a makhorka tobacco (N. rustica) stock synthesizes nicotine only when the stock possesses a well-developed leaf surface. If only the root system of the stock is present, or if the leaf surface of the scion is predominant over that of the stock, nicotine is not synthesized.

In grafts of makhorka tobacco (N. rustica) on non-nicotine-containing plants, tomato (S. lycopersicum), potato (S. tuberosum), fragrant tobacco (N. affinis), or solanum (S. Nigra), the scion loses its ability to synthesize its native alkaloid, nicotine. Nicotine synthesis fails to occur even when only the root system of the stock is present.

Tomato (S. lycopersicum) and potato (S. tuberosum) grafted onto makhorka tobacco synthesize nicotine, an alkaloid not native to them. This occurs even when only the root system of the stock is present. The disappearance of nicotine in cuttings from the tomato scion (graft of tomato onto makhorka tobacco) confirms the supposition that nicotine is formed in the roots of makhorka tobacco.

In our opinion, further study with fragrant tobacco makhorka tobacco grafts will aid in clarifying the nature of conversions of nicotine in leaves or stems of fragrant tobacco, underlying which are the most intimate aspects of metabolism of nicotine-containing and nicotine-free plants.

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CHANGES IN GROWTH AND DEVELOPMENT OF PEACH INDUCED BY SUBJECTING GERMINATING SEEDS TO LOW TEMPERATURES

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It is well known that young, still undifferentiated tissues (growing points, embryos) have a greater plasticity than mature tissues [1-3].

Many workers [4-8] have also shown that by subjecting soaked seeds to changing temperatures in the range from 0 to −2°, and from +18 to +25°, it is possible to increase cold resistance, induce earlier flowering, and increase yield capacity of thermophilic crops.

In the light of these findings we performed experiments in which imbibed peach seeds were exposed to alternating warm and cold periods in an effort to hasten fruit bearing and increase cold resistance.

In the spring of 1954, barely soaked seeds of Ak-Shaftalyu peach which had been collected from one tree were given the following temperature treatments: 1) Control—planted at once in humus pots and then in the ground; 2) planted in humus pots and then subjected to the following temperature regime: 24 hours at 5°, 48 hours at 20°, 24 hours at 3°, 48 hours at 20°, 24 hours at −1°, and 24 hours at 20°. Total time of exposure to temperatures from 6° to −1° was 72 hours; 3) planted in pots, then subjected to the following temperature regime: 12 hours at 5°, 24 hours at 20°, 12 hours at 3°, 24 hours at 20°, 12 hours at 1°, 24 hours at 20°. Total time of exposure to low temperatures was 36 hours.

After the cold treatment the pots containing the seeds were placed in sand boxes and kept at 20° for seven days, and on April 11, 1954, the seeds were planted in the ground.

As is well known, in 1954 severe frosts in Uzbek killed thousands of acres of orchards. The controls were killed at this time, while the treated plants survived the winter; in the latter only the tips of shoots which had not completed their growth by November and had not undergone hardening suffered injury.

Gross observations of plants indicated that plantings from cold-treated seeds at first grew slowly, but subsequently began to develop more rapidly than the controls; measurements were not made in the fall of 1954, however. In the winter the controls all died, and therefore comparisons could only be made among the experimental groups (Table 1).

Measurements made after 16 and 28 months (August 24, 1955 and August 25, 1956) showed that by all criteria employed, growth was better in plantings from seeds exposed to a 72-hour cold period than in those from seeds exposed to a 36-hour period.

The lack of a control constrained us to repeat the experiment in 1955, though in somewhat modified form. Kernels of stratified seeds were kept in distilled water for three days, the water being changed daily. The outer integument was then removed and the seeds placed in sand for swelling; during this time the control group was kept at 20° and the experimental group subjected to the following regime: 24 hours at 3°, 24 hours at 20°, 24 hours at 1°, 24 hours at 20°, 24 hours at −1°, 24 hours at 20°. After this treatment the seeds were planted outside.

TABLE 1. Growth of Aerial Portions of Beech Saplings Grown From Seeds Which Had Been Cold-Treated and Set Out on April 11, 1954

Date of measurement	Duration of cold treatment, hr	Tree height, m	Tree circumference, cm	Total branch length, m	Leaf surface, m²
8/24/55	36	2.14	6.0	35.0	1.75
	72	2.62	9.5	47.0	2.20
8/25/56	36	3.15	20	101.0	2.0
	72	3.51	26	225.0	3.0

As Table 2 shows, at the end of the first year the experimental plants on the average lagged behind the control plants in respect to all the growth criteria employed. In the following year, 1956, they had almost caught up with the controls, and in the third year, 1957, they had overtaken the controls. The flowering behavior was especially significant. The number of flowers on one tree varied from 0 to 50 in the control group, and from 60 to 700 in the experimental group.

In addition to gross observations, biochemical measurements were made on individual plant organs (Table 3).

The overall trend of two years' data clearly indicates that chemical composition varies little with experimental treatment. Buds of plants from seeds given a 72-hour cold treatment had a somewhat higher sugar

TABLE 2. Growth of Aerial Portions of Peach Saplings Grown from Seeds Which Had Been Cold-Treated and Set Out in the Spring of 1955 (av of 10 plants)

Treatment	Date of measurement	Tree height, m	Tree circumference, cm	Total branch length, m	Leaf surface, m^2	No. of flowers per plant
Exptl.	8/24/55	0.96	2.5	2.7	0.02	–
Control	8/24/55	2.05	2.6	5.3	0.05	–
Exptl.	8/25/56	1.65	9.4	26.3	0.18	–
Control	8/25/56	1.92	9.9	26.1	0.22	–
Exptl.	8/25/57	3.30	33.0	36.0	–	251
Control	8/25/57	2.17	30.0	23.0	–	20

TABLE 3. Content of Various Chemical Substances in Peach Plants Planted in 1954 (in % of absolute dry wt; av over two years)

Cold exposure time	Buds		Leaves	One-year-old shoots	Stem wood	Roots	Total
	leaf	fruit					
			Total sugar				
36	4.3	8.7	3.1	2.2	3.1	2.3	23.7
72	4.7	9.4	2.9	2.6	2.0	2.1	23.7
			Starch, hemicelluloses, pentosans				
36	5.0	4.1	–	10.4	–	12.9	32.4
72	5.3	5.8	–	6.8	–	12.7	30.6
			Total nitrogen				
36	6.3	4.3	1.7	1.3	0.9	1.2	15.7
72	6.2	4.3	1.7	1.3	1.0	1.2	15.7
			Total phosphorus				
36	1.4	1.0	0.6	0.5	0.4	0.6	4.5
72	1.3	1.0	0.6	0.6	0.5	0.7	4.7

TABLE 4. Chemical Composition of Peach Plants Planted in 1955 (av over one year)

Treatment	Leaf buds	Leaves	One-year-old shoots	Roots	Total
		Total sugar			
Exptl.	6.3	4.5	2.6	2.1	15.5
Control	5.2	3.7	2.2	1.7	12.8
		Starch, hemicellulose, pentosans			
Exptl.	3.3	–	15.4	22.4	41.1
Control	1.6	–	4.2	4.5	10.3
		Total nitrogen			
Exptl.	5.5	1.7	1.4	1.2	9.8
Control	4.4	1.6	1.0	1.1	8.1
		Total phosphorus			
Exptl.	1.5	0.6	0.5	0.6	3.2
Control	1.4	0.6	0.4	0.5	2.9

and starch content than those of plants from seeds exposed to low temperatures for 36 hours. In one-year-old shoots more starch had been accumulated when exposure time was 36 hours.

A study of plants of the 1955 experiment revealed a marked influence of temperature treatment (Table 4).

As these data show, the carbohydrate, nitrogen, and phosphorus content of plants subjected to cold was higher than that of control plants. Starch and sugar accumulation was especially pronounced.

The higher level of reserve materials in germinating seeds which have been subjected to alternating warm and cold periods promotes extensive accumulation of nutrients; this in turn enables the tree to lay down a large number of buds at earlier periods, and thus the yield capacity of the tree is enlarged.

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INFLUENCE OF TEMPERATURE CONDITIONS ON THE DEVELOPMENT OF FLOWER BUDS AND FRUITS

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Fruit trees react in different ways to frost depending on winter conditions and, above all, on the time at which the cold period sets in [1, 2].

After the severe winter of 1934, flowering in apple and pear was sparse; in June a new flowering began of buds which had been formed in a normal manner in 1933. A large number of the flowers were, however, hyperpetaloid, with twenty petals in place of five. We observed belated hyperpetaloid flowers in Lyubimits Klapp pear after every severe winter.

In 1956, after a cold winter, flowering of quince was retarded and did not begin until June 10. The flowers were reminiscent of polyploid roses. In 1952 and in 1958, hyperpetaloid flowers were observed in apricot (Figure, A). Sometimes, after severe frosts, which inhibit bud opening the following spring, filaments in floral buds were converted into petals, as observed in quince in 1954 (Figure, B).

The delay in flowering which follows severe winters, even when spring conditions are fully favorable, is dependent on the extent of metabolic transformations of nutrient materials after emergence from dormancy.

In the fall, floral buds of fruit trees contain no starch. With cessation of growth, starch is accumulated in the bud, being first deposited in the bud scales, at the base of the bud, and only in the middle of the winter in the stamens, the pistil, and the stigma. The appearance of starch in flowers indicates that the dormant period is nearly at an end. Usually, immediately after the appearance of starch plasmodesmata arise, first in the petals and then at the base of the bud.

Frost retards completion of floral bud differentiation, and the absence of warm weather, emergence from dormancy. With the advent of mild spring weather, dormancy is terminated and differentiation is completed. Bud development at high temperature differs from that at moderate (normal) temperatures.

In most varieties, delayed flowering may be observed both after severe winters and after warm ones, which are not infrequent in Kuban. In a warm winter, differentiation of floral buds is rapid, and by December they are fully capable of opening, but metabolic transformations are retarded, which prevents growth inception (Table).

As the data show, in 1952, the winter was mild and the level of floral bud differentiation in Gayar 9 peach was high. Sugar content was high and remained so throughout January; starch content, however, was low in December and even in January, but showed a pronounced increase in February. Spring flowering was delayed by the slow conversion of sugar into starch during this mild period. Flowering and fruiting were vigorous this year.

The same pattern was noted for the 1954-1956 period, in which differentiation occurred at the normal time, but starch formation was retarded, with the result that emergence from dormancy and inception of flowering were delayed.

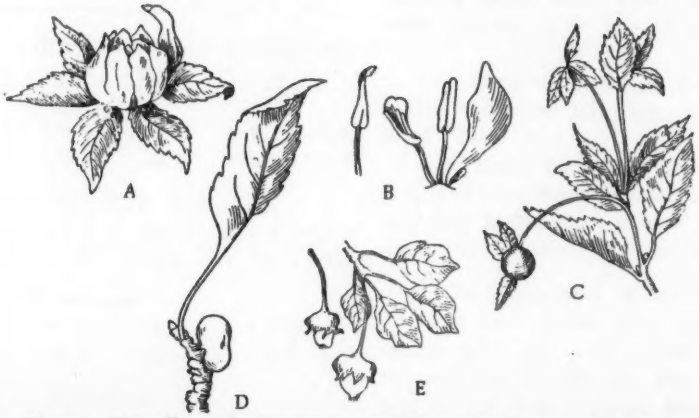

Figure. The effect of temperature conditions on flower and fruit formation.

TABLE. Levels of Biochemical Development and of Floral Bud Differentiation in Gayar 9 Peach in Different Winters

Years	Temperature											Amt. according to 5-point system				Level of flower formation, 5-point system		
	average				maximal			minimal				of starch		of sugar				
	Nov.	Dec.	Jan.	Feb.	Nov.	Dec.	Jan.	Feb.	Nov.	Dec.	Jan.	Nov.	Dec.	Nov.	Dec.	Jan.	Nov.	Dec.	Jan.
1952-1953	7.3	1.2	2.2	2.6	16.8	14.4	19.2	14.7	− 4.3	−13.8	−11.6	3	4	4	3	3−	4−	5	5
1953-1954	−1.1	−2.3	−4.4	−12.6	11.7	11.9	−6.6	3.6	−21.4	−25.2	−27.2	4	5	4	4	4	3+	3+	3+
1954-1955	7.6	2.6	3.7	6.2	23.7	17.7	12.9	20.6	− 3.7	− 7.1	− 7.2	1	4	4	4	3−	4−	4+	5
1955-1956	4.8	1.3	3.2	− 9.9	17.2	13.5	15.2	13.3	− 7.4	−16.0	−24.0	3	4	3	3	2	4−	5	5
1956-1957	3.0	0.1	−2.8	4.2	19.9	18.6	13	18.9	−14.1	−12.8	24.7	2	4	4	3	3−	4	4	5

In cold winters, flower development is retarded, in spite of the fact that starch is formed early—in November and December in peach, apricot, and cherry, at the base of the bud, in the bud scales, and in the petals.

Normal development of floral buds in November and December takes place at an average daily temperature of from 2 to 4°. Delayed development occurs at considerably higher average daily temperatures, from 7 to 10°, with maxima approaching 20°.

Oknina and Barskaya [3] demonstrated the occurrence of multinucleate cells in dormant tissues, which ensures a vigorous spring growth. We explain the formation of hyperpetaloid flowers, which is associated with belated flowering, in the following way: During a warm winter flowers become highly differentiated, but do not pass through a dormant stage, and it is in this condition that there is an abundant accumulation of nuclear material, which is related to the inhibition of growth. As a result, at high spring temperatures abnormal growth occurs: In place of filaments, petals appear, and hyperpetaloid flowers are formed. At the tips of the petals, abnormal stamens may be seen.

It is easy to distinguish belated flowering from secondary flowering by the character of the flowers. We should differentiate between these two phenomena. Belated flowering involves buds laid down at the normal time which have passed through a dormant period (winter development). Secondary flowering, which may occur at any time during the growing period, involves buds which have not undergone winter development. Fruits are also formed after secondary flowering, but as a rule they are even more abnormal than those produced from a delayed flowering.

At Severskaya, we found an old apple tree of a variety indigenous to the area which flowers three times a year. It was not possible to determine the variety. The first flowering is at the normal time. The second flowering, which is in June, we consider belated. Fruits from the first flowering ripen in July, and at this time there are both mature fruits and immature ovaries on the tree. The third flowering, in September, represents buds laid down in the current year which have not undergone winter development, and as a result the flowers are frequently abnormal and the fruits are elongated in form and fail to ripen before frost.

Apples in this region behave as do peaches and cherries in Ceylon or the Antilles where, according to Hedrick [4] and Kostanten [5], ripe fruits, immature ovaries, and flowers may be seen on a tree at one time.

In order to induce secondary flowering in Vladimir cherry, on August 8, 1940, we burned the leaves. As a result, buds in the middle portion of one-year-old shoots began to develop. Branched shoots resembling inflorescence axes were formed, and in September normal flowers with sepals intermediate in form between leaves and sepals appeared (Figure, C).

In the 1951-1953 period, complete removal of all leaves from Muskatnaya Prazhskaya cherry on August 15 induced the growth of the middle buds on one-year-old shoots to form floral axes on which flowers appeared in September. The sepals were of intermediate form, and resembled leaves even more than in 1940. In 1956, an extensive secondary flowering was observed in strawberry. Among normal fruits there were fruits in which sepals were disposed in concentric circles over the entire length of the fruit.

Unusual temperature conditions are the decisive factor in the appearance of flowers, ovaries, and fruits which are atypical for the given race or variety.

During a cold snap in March of 1940, in the Krasnodar area, apple buds were killed. No flowering occurred. In August, dwarf apples were formed at the site of the bud scars at the base of the years growth. These apples did not exceed 1.5 cm in diameter (Figure, D). They were of a color characteristic for the variety. The core was undeveloped. The pulp, which was of a woody texture, was composed of small cells, but annular vessels characteristic of normal fruits were present. Such dwarf apples were found in 1950 and in 1956 after the severest injury to the floral buds.

How widely aberrant fruits which develop under abnormal temperature conditions may be is seen in an indigenous pear which we discovered in the Krym district. In winters warm for Kuban (for example, 1947-48, 1952-53, 1956-57), floral buds emerge from dormancy at different times. Flowers which develop during a hot period produce abnormal fruits which are mistakenly called vegetative. In the summers of 1948, 1953, 1955, and 1957, there were very many such fruits produced by the indigenous pears (Figure, E). This phenomenon occurs throughout the south after warm winters, most often in varieties with a long dormant period. Thus, a disturbance of normal winter and summer temperature conditions leads to the formation of abnormal flowers and fruits.

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DIAGNOSTICS OF THE DEMAND BY TOMATO PLANTS FOR WATER BASED ON THE TRANSPIRATION AND THE CONCENTRATION OF CELL SAP OF THE LEAVES

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The possibility of determining the demand by plants for water not only by soil moisture, but also by the condition of the plant itself, came to the attention of physiologists long ago. The development of the methods of using plants as qualitative indicators to determine the need for water can, through the economical use of irrigation, result in high yields.

The physiological indicators characterizing the water capacity of the plants has been established for grain crops, cotton, sugar beet, and several other agricultural plants, and it is possible to use this in the determination of norms and dates of irrigation [1-8].

This question has been studied only slightly in reference to vegetable crops. Investigations in this direction were carried out only in the conditions of southern RSFSR, where the specific relationship between the concentration of the cell sap of the leaves and the soil moisture was established. On the basis of these investigations, recommendations on the use of the concentration of the cell sap as a measurement of the demand of vegetable plants for water were offered [9, 10]. This question has not been studied at all for conditions of the zone of inadequate moisture, the Central nonchernozem belt, where irrigation also is one of the important factors in the area of the productivity of vegetable crops.

In connection with this, in 1957 we carried out investigations on the effect of changes in the concentration of the cell sap and the transpiration stress of the leaves of tomatoes in relation to the changes in the water regime of the soil in which they were growing. The goal of the investigations was to establish for the conditions of the zone of inadequate moisture, the size of the indicators of the concentration of cell sap and the transpiration stress of the leaves of tomatoes under optimal soil moisture conditions and with insufficient moisture. Such data would make it possible to resolve practically the questions of the determination by physiological indicators of the water requirements of tomato plants.

The experiments were carried out on the experimental base of the Scientific Research Institute of Vegetable Growing near Moscow in containers in a vegetation shed and in the open ground with varieties Gruntovyi Gribovskii and Krasnoznamennyi. The containers were 23 x 23 cm and the original weight of the soil in each container was 7200 g. The soil was a sod with an added 10% of compost. The original moisture was 20.3% with a field capacity of 72.6% (measured by laboratory method).

In the process of the following work, the soil moisture in the containers was held constant by means of daily watering: in the first variant at a level of 40-50%, in the second at 60-70% and in the third at 80-90% of full field capacity. Soil moisture of 60-70% of field capacity is optimal for growth and development, 40-50% is insufficient, and 80-90% is excessive.

In the open ground the physiological changes in the naturally accumulated moisture conditions in the soil were studied in order to establish in a parallel manner with vegetation experiments the size of the transpiration stress and the concentration of the cell sap with optimal soil moisture and with an inadequate supply of water.

There were seven repetitions with variety Gruntovyi Gribovskii and three with variety Krasnoznamennyi.

Insofar as the experiments with both varieties were carried out according to a single scheme and the results obtained were similar, we will give only the data for variety Gruntovyi Gribovskii.

We made careful observations in the vegetation containers on the growth and development of plants under different moisture regimes, on the accumulation of dry substance in the aerial part and root system of the plants, and calculated the yields and the outflow of water in transpiration, etc.

We will not present these data in the present work. We will only note that growth and development proceeded more intensively in plants with optimal soil moisture (60-70% of field capacity), than in the variants with inadequate or excessive moisture, as was expected. The yield of fruits in this variant was highest: For variety Gruntovyi Gribovskii with 40-50% of field capacity, the yield was 563.4 g, with 60-70% it was 1085.6 g, and in the variant with excessive moisture it was 499 g per plant. The dry weight both of the vegetation mass and of the root system of the plants also was highest in the optimal variant with soil moisture of 60-70% of field capacity.

When studying the transpiration stress in tomato plants, we first tried to select the most suitable methods for its determination in work with this crop.

TABLE. The Effect of Soil Moisture on the Size of the Transpiration Stress of Tomato Leaves and Their Concentration of Cell Sap (in vegetation containers)

Time of taking samples	Phase of growth	Air temperature, °C	Rel. humidity, %	Transpiration stress of leaves, atmos			Conc. of cell sap, %		
				Soil moisture as % of field capacity					
				40-50	60-70	80-90	40-50	60-70	80-90
7/6	Beginning of formation of fruits on the first flower	18.2	85.6	11.4	9.0	4.7	10.3	8.3	7.2
7/13	Formation of fruits on the first flower	21.2	81.4	9.7	8.1	6.7	10.1	9.2	7.4
7/24	Growth of fruits	25.3	70.3	11.4	10.5	9.7	11.2	9.7	9.4
8/23	Beginning of ripening of fruits	26.2	53.8	16.0	12.9	12.9	10.3	8.8	7.5
9/4	Fruit bearing	24.2	79.2	12.9	9.7	–	9.9	8.3	7.2
9/24	Fruit bearing	18.6	76.4	10.5	10.5	–	9.9	8.3	–
	Average size			12.0	10.1	8.5	10.3	8.8	7.7

In comparing the compensation method of measuring the transpiration stress of the leaves [4] and the flow method and gradient tube method [8], we came to the conclusion that in work with tomatoes in our conditions the most favorable is the flow method. The compensation and gradient tube methods are not very suitable for work with tomatoes, inasmuch as in mixing leaf cuttings in sucrose solutions, as is specified in carrying out the analyses see[4, 8], the concentration often changes so little that the differences between the original solution of sucrose and the solution with the cuttings is almost impossible to see with the help of a refractometer or gradient tube.

Using the flow method, it is easier to establish these differences inasmuch as even with small differences in the concentration of the two solutions the flow is quite clearly visible and it can always be stated objectively if the concentration of the original sucrose solution increased or decreased when cuttings from leaves of the study plants were placed in it. We also obtained similar data in work with cabbage and cucumbers.

The measurement of the transpiration stress (and also of the concentration of the cell sap) was carried out periodically during the second half of the vegetation of plants, always at 10 A.M., when these indicators depend more on the soil moisture and less on changes in meteorological factors (temperature and relative humidity of the air, isolation from the sun, wind, and others). The samples were usually taken with samples from the first leaf under the first flower, the first and second lobes, with 15-20 cuttings from leaves of all plants in the experiment.

We observed sharp changes in relationship to the soil moisture (see Table) based on the transpiration stress of the leaves in the first period of vegetation (from setting out the seedlings up to the beginning of the ripening of the fruits).

Under optimal moisture conditions (60-70% of field capacity), the transpiration stress was equal on the average to 9-10 atmospheres. A decrease in soil moisture down to 40-50% of field capacity caused an increase in the transpiration stress to 10-11 atmospheres; with excessive moisture, a sharp decrease was observed.

In the second half of the vegetation of plants—in the period of growth and ripening of the fruits—the transpiration stress increased in all variants, whereupon its size depended less on the soil moisture than in young plants. Sometimes these differences were not observed at all. Such an effect, apparently, would make the use of transpiration stress as an indicator in diagnostics of the requirement of tomatoes for water difficult because under such conditions it would be hard to establish a specific size of transpiration stress by which the need or lack of need for water could be judged.

The concentration of cell sap of the leaves was determined by the field refractometer according to a method improved somewhat by us (using a filtration of the cell sap through a paper filter, which made it easier to observe the cloudy sap of the leaves of tomato or other vegetable crops in the refractometer).

In studying the changes in the size of the concentration of the cell sap of tomatoes during vegetation under different soil moisture conditions, clearer differences were obtained than for the transpiration stress (see Table). Under an optimal soil water regime (60-70% of field capacity) the concentration of the cell sap was in the range from 8-9%. With a decrease in the soil moisture down to 40-50% of field capacity, the concentration of the cell sap increased to a level of 10-10.5%. With excessive soil moisture, the concentration of the cell sap decreased to 7.0-7.5%. In the open ground, where there was plentiful soil moisture during the vegetation period (on the average its moisture level was 87.1% and varied from 74.2 to 100% of field capacity), the average size

for the concentration of cell sap was 7.9± 0.22%, that is, it was similar to that obtained in the vegetation shed.

We observed the clear effect of changes in soil moisture on the concentration of the cell sap in the leaves throughout the entire vegetation of the plants. This shows the possibility of using the concentration of cell sap of tomato leaves in determining the need for water by plants of this crop.

An increase in the concentration of the cell sap in the leaves to 10-10.5% is a signal for inadequate soil moisture and for the demand by the plants for water.

A further stage in the present work would be to make more precise the absolute sizes of the concentration of the cell sap for open ground conditions with optimal soil moisture and inadequate moisture, which would make easier the practical use of this indicator in diagnostics of water requirements.

Thus, as a result of the investigations that we carried out in the conditions of the zone of moisture deficiencies, a specific relationship was established between the water supply in tomato plants and the physiological processes characterizing the condition of their water regime (dynamics of the transpiration stress of the leaves and their concentration of cell sap). The sizes of these indicators by which the demands of the plants for water can be judged were established. The concentration of the cell sap is the best indicator in diagnosing the demand of tomato plants for water.

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THE ABILITY OF SEEDS TO ENDURE ULTRA LOW TEMPERATURES

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The work of a number of investigators has established that the dry seeds of various plants endure without damage such ultra low temperatures as −90°C [1-8], −250° [9], −270° [5-10] and even temperatures only 0.05-0.008° higher than the absolute zero [11]. However, the resistance to frost of seeds in a soaked condition appears to be low [1-4, 10] and a number works showed that the higher the degree of soaking of the seeds, the lower is the resistance [7, 8, 12-17]. Thus, according to the data of Stuckey and Curtis [12], wheat seeds did not germinate if they had been frozen for a period of 5 hours at a temperature of −20° to −25° with a concentration of water in them of more than 32%; if the same seeds during a similar freezing contained less than 20% water, based on the dry weight, then 10% germination was observed. The data of Lockett and Luyet [7] showed that wheat seeds with a water content of 19, 23, and 25% had a corresponding germination rate of 92, 38, and 0% after freezing at −190° for a period of 2 minutes. Similar results were obtained with oat seeds [13], corn [14, 15], clover [16], and lucerne [17].

On the basis of the results of these works, one can come to the conclusion that the seeds of various plants are capable of enduring freezing at temperatures of −20° and lower, but only if the concentration of water in them is not more than 20% of the dry weight (that is, not more than double the quantity of water contained in air dry seeds) or not more than 30% in relation to the dry weight for some embryos [7]. With further soaking of the seeds, their frost resistance rapidly decreases and even with a concentration of water in the seeds of 30%, they do not retain their frost resistance at −20°. However, completely swollen and even sprouted seeds [5, 10, 16] or sprouts [8] are capable of being held at very low temperatures (even −190°) if they are previously dried to the indicated level.

In all of the works listed above, the seeds were frozen immediately at the indicated low temperatures. From the numerous work on the reasons for cell mortality associated with freezing, it is known that such rapid decrease in temperature allows the formation of ice inside the cells, which always leads to their death. Therefore, we can suppose that the death of swollen and sprouted seeds in these experiments took place as a result of the formation of ice in their cells.

We also know that with a slow lowering of the temperature, ice is formed in the intercellular spaces taking away the water from the cells and resulting in their dehydration, which is similar to that taking place in drying. Such an extra cellular formation of ice is not as dangerous to the cells as the internal cellular formation and it is less dangerous in proportion to the resistance of the cell to drying [18, 19].

Insofar as the swollen seeds and even those that have begun to sprout can be immediately dried without loss of their germination, which suggests the resistance of their cells to dehydration, then it is natural to suppose that they can be resistant also to the extracellular formation of ice. The gradual dehydration of cells taking place with a decrease in temperature can make them still more resistant to low temperatures by preventing the formation of cellular ice as a result of drying the seeds.

On the basis of what has been said, one can expect that the seeds will be able to endure very much lower temperatures with a slow decrease in temperature corresponding to the rate of exit of water from the cells than with a rapid decrease in temperature. This supposition is based also on the results of the experiments by Tumanov and Krasavtsev [20] and Sakai [21] with the branches of woody plants, which endured ultra low temperatures of −190° and −250° under conditions of a slow decrease in the temperature.

Based on this, we established a number of experiments with wheat, rye, and corn seeds. The seeds were placed in beakers with water for one to two days for soaking at room temperature, after which they were frozen in test tubes or boxes at −3° (in several experiments the seeds were kept from two to six days in a moist atmosphere at +2° after swelling). Beginning at −3°, the temperature was gradually lowered to −6° so that the normal duration of the seeds at minus temperatures varied from seven to nineteen days in the various experiments. After this the temperature in the refrigerator was gradually increased (after one to two days) to +2°, or the test tubes with the seeds were changed directly to +2°. The thawed seeds were spread out on wet filter paper, the ends of which were placed in water. They were placed in the laboratory in light at +18° and +20° and the germination of the seeds was calculated after several days.

In one of the experiments, seeds of winter wheat of three varieties were taken: Ul'yanovka, Moskovskaya 2453, and Lyutestsens 329, containing 55-60% water after swelling. After freezing to −60°, 62, 54, and 47% of the seeds germinated. In the other experiment, 98% of

the seeds of winter wheat, variety Moskovskaya 2453, and 97% of summer variety Lyutestsens 62, which contained 70 and 54% water, respectively, germinated. However, the germination of seeds after such freezing did not proceed simultaneously: The greatest part of them had already germinated in the first two or three days, and the remaining part in the following five to ten days. For sprouts from those held with the germinated seeds several divergences from normal growth were also noticed afterwards.

After having obtained these results, we decided to go further and freeze soaked seeds down to −190° and −250°. At the beginning the seeds were gradually frozen down to −60° (as in the previous experiments) and then transferred into test tubes in liquid nitrogen (−190°) for a day, and in one experiment from the nitrogen into liquid hydrogen (−250°) for four hours.* After this the test tubes with the seeds were transferred back into the container with a temperature of −60°, and then the temperature was raised to +2°.

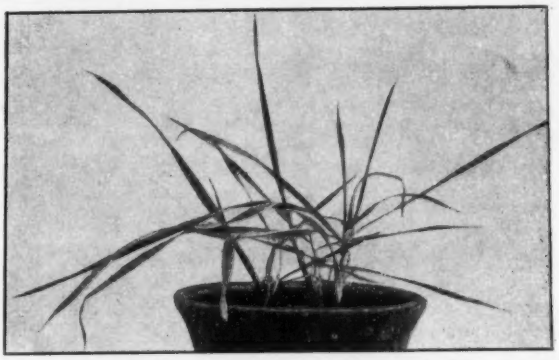

Figure. Rye plants, variety Gibridnaya, obtained from seeds after freezing them in a soaked condition down to −250°.

Rye seeds (variety Gibridnaya) were used for these experiments and one experiment was carried out with Moskovskaya 2453 wheat. After freezing down to −190° from 9 to 71% of the seeds in various variants survived and after −250° from 22 to 43% survived.

Here it is necessary to stress that the high percent of survival was also observed in those variants in which the seeds had a high concentration of water; Thus, 71% of the seeds with a concentration of water of 69% sprouted after −190° and 43% with a concentration of water of 68% after −250°.

In this manner, the swollen seeds of wheat and rye which contained water up to 70% of the absolute dry weight gave a higher percent of germination after freezing down to −190° and −250°. However, this was obtained only in those experiments in which the seeds were under a 2 to 3 cm layer of water at the time of swelling, which formed a certain degree of anaerobiosis and retarded their sprouting. If the same seeds were soaked only in a small quantity of water, which would aid their sprouting, then after freezing a significantly lower number of seeds would survive.

Thus, in one of the experiments seeds were soaked for one day under a layer of water and were frozed down to −190° in a small quantity of water. In spite of the almost similar concentration of water in the seeds of both variants prior to freezing (82 and 79%), 39% in the first variant germinated and 12% in the second; this is connected with the fact that in the second variant the seeds already had started to sprout but in the first were not successful. Even more sharp was the difference of these two variants in swelling if the period was stretched out for two days, in which case the seeds in the second variant had already sprouted (up to 10 mm) and in the first only started to sprout. In this case 27% of the seeds in the first variant germinated, but none in the second. When the seeds were soaked under a layer of water for a period of three days, they sprouted and contained 113% water, that is, significantly more than when soaked one day in a small quantity of water (79%). However, in both cases approximately the same quantity of seeds germinated (10 and 12%) and in both variants the seeds had a similar degree of sprouting. These results led to the conclusion that the resistance of seeds to a slow rate of freezing depends not so much on the concentration of water in them as on the intensity of the growth process in the embryos.

It is possible that the decreased resistance of the seeds to freezing after sprouting is connected with the decreased resistance of the cells of the embryo to dehydration [5, 8] and, consequently, also their resistance to the dehydrating action of ice external to the cell. The same resistance to a gradual freezing of the swollen, but not sprouted, seeds can explain the resistance of their cells to any dehydration. However, the experiments that we carried out with corn seeds showed that this was probably not the case. The corn seeds soaked under water for one day and containing 50% water after this were frozen gradually down to −60° in one of the experiments along with wheat seeds. After thawing, not one of the corn seeds sprouted, while the soaked corn seeds underwent reverse dehydration very well.

It is possible that with gradual freezing continuing for a week or more, processes of hardening take place in the soaked seeds of wheat and rye, which increase the resistance of their cells to the action of ice [22], but these processes are poorly expressed in the cells of the seeds of southern corn plants, and, therefore, the seeds do not attain very great frost resistance. The results that have been presented show that we still know very little about the frost resistance of seeds of various plants

* The freezing in liquid hydrogen was carried out in the Institute of Physics Problems of the USSR Academy of Sciences by scientific co-worker A. S. Borovik-Romanov, to whom the author extends his thanks.

in a soaked condition and further study of this question is necessary. It is interesting not only from the theoretical side, but also from the practical side as, for example, in connection with the proposal of S. V. Krylov on the winter planting of field and garden crops [23].

Besides this, our observations of plants from seeds subjected to hard freezing while in a soaked or sprouting condition show that this action does not take place without an effect on further growth, at any rate during its first phases. Therefore, it is possible that the use of such methods on seeds opens new perspectives for increasing the frost resistance of plants and selection of new frost resistant varieties.

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SOME FEATURES OF THE FORMATION OF POTATO CROPS UNDER CONDITIONS OF IRRIGATION IN THE SOUTHEAST

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Moisture deficiencies in the soil and high temperature in the vegetation period limit potato yields in the conditions of the dry regions of the Southeast. In connection with this, there arises a need for irrigation directed at obtaining high and resistant yields of this valuable crop. In 1955-56 the authors carried out experiments in the zone of the third sluice of the V. I. Lenin Volga-Don canal under commercial conditions with winter and vegetation period irrigation of spring plantings of potato (variety Lorkh).

The experiment had five variants. The first variant was on a dark-chestnut soil with sets distributed at 70 x 50 cm; all remaining variants were on light-chestnut soil with sets at 70x70 cm. The irrigation scheme by variants was as follows:

Variant No.	Irrigation scheme
1	Winter irrigation, 2000 m^3/hectare. Vegetation period irrigation by sprinkling: first—400 m^3/hectare, second—200 m^3/hectare, third—100 m^3/hectare.
2	Without winter irrigation. Irrigation during the vegetation period same as Variant 1.
3	Winter irrigation, 1500 m^3/hectare. Irrigation during the vegetation period: first and third sprinklings—200 m^3/hectare, second—in trenches 500 m^3/hectare.
4	Winter irrigation, 1200 m^3/hectare. Irrigation during the vegetation period same as in Variant 3.
5	Winter and vegetation period irrigation same as Variant 1.

Winter irrigation appeared to have a favorable effect on the temperature and moisture of the soil. For example, in Variant 5 the reserves of moisture prior to setting out potatoes was 800-1000 m^3/hectare and even at the end of the vegetation period was 250-350 m^3/hectare more than in Variant 2, with no winter irrigation (Fig. 1). At the same time that the soil temperature in Variant 2 reached 28-30° in the zone of tuber formation (at a depth of 15 cm), it was 4-6° lower in Variant 5 and did not exceed 26°.

The sharp difference in the temperature and moisture of the soil on the fields with winter irrigation and without (control fields) clearly showed in the growth of the potatoes. The productivity of photosynthesis on the winter irrigated fields was on the average 20-30% higher (Fig. 1) but the yield of tubers was approximately twice as high (Table 1).

The field observations of the dynamics of the formation of the yield made it possible to explain several features of the productivity of photosynthesis and growth of potatoes under winter and vegetation period irrigation. In Fig. 1 the change in the productivity of photosynthesis of potato, based on these observations, is shown for the vegetation for Variant 2 and 5 in connection with the temperature and moisture of the soil.

As the graph shows, the minimal figure for the productivity of photosynthesis of the potato is observed six-seven days later than the minimal figures of the moisture reserves in the soil. The potato, just as other crop plants, wilts relatively slowly when the soil dries and reestablishes its photosynthetic activity when the soil is moistened.

A similar relationship of the productivity of photosynthesis to the soil temperature was not explained by experiments that were established. We can suggest that in the dry regions of the Southeast the moisture regime has a decisive influence on the productivity of photosynthesis of potatoes, while a much lower influence is exerted by the soil temperature. With sufficient moisture reserves in the soil, attained by timely and correct irrigation, the productivity of photosynthesis can reach 12-13 g/m^2 (Fig. 1), which exceeds the corresponding indicators (about 9 g/m^2) obtained by Stroganova [1] on the fields of the Scientific Institute of Potato Growing.

The question of the effect of the productive soil moisture on the growth of the potato and on photosynthesis is also very interesting, but, unfortunately, little studied.

At the present time, we can consider as established, on the basis of experiments with several crop plants, that the rate of growth of the plants increases irregularly with an increase in the soil moisture from the bound moisture up to the minimum moisture capacity; there exists a critical moisture level, on improvement of which the intensity of the growth increases intermittently [2, 3]. On the other hand, the growth of plants is sharply retarded with a decrease in the soil moisture below this critical level. The indicated position is graphically illustrated in Fig. 2, where the productivity of photosynthesis and the change in the dry weight of the potatoes for the early

TABLE. The Relationship of the Yield of Potatoes to the Leaf Area

Variant	Max. leaf area, 10^3 m²/ha	Dry wt., ton/ha	Green wt., ton/ha		Leaves: wt. of aerial part
			aerial 8/20	tubers	
1	31.3	7.20	20.60	17.76	1:1.45
2	27.5	3.80	18.25	6.76	1:1.51
3	25.8	5.51	16.46	12.92	1:1.65
4	23.7	4.41	14.27	11.68	1:1.58
5	21.9	5.03	14.40	12.41	1:1.50

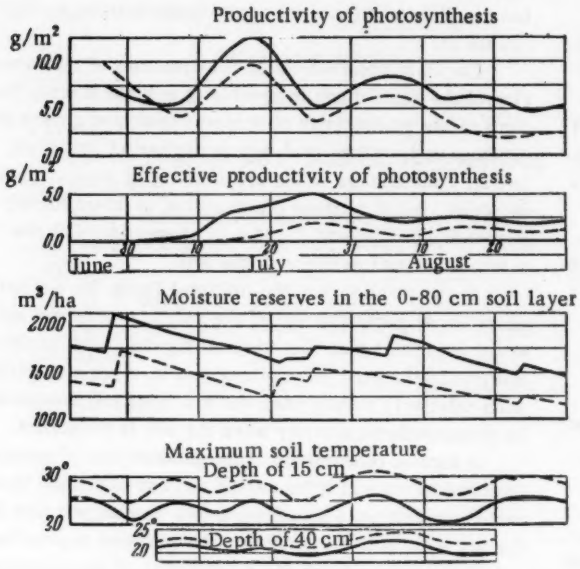

Fig. 1. Dynamics of the change in production of photosynthesis of the potato for winter irrigation and control (without winter irrigation) field in relation to the temperature and moisture of the soil. Solid lines denote biological growth indicators and soil characteristics for winter irrigated fields (Variant 5); broken line—control field (Variant 2).

stages of tuber formation are shown in relation to the reserves of productive moisture in the root layer of the dark-chestnut soil (80 cm). It follows from Fig. 2 that when the soil moisture is increased in the range of 72-68% of the minimum moisture capacity, the productivity of photosynthesis of the potato is decreased from 11 to 7 g/m², that is, by 36%, and the daily growth in dry weight by 25%. A decrease in the productivity of photosynthesis at this level of soil moisture and at any other level, other than the minimum moisture capacity (72-68%), does not exceed 5-10%. It is interesting to note that with a soil mixture corresponding to a moisture level retarding growth, the irrigation of all organs of the potato is changed not more than 3-5%.

In order to obtain high yields of agricultural crops, including potatoes, it is necessary to use winter irrigation to ensure that spring soil moisture reaches the level of the minimum capacity, and that the moisture does not fall below the level of inhibiting growth during the vegetation period. To guarantee the latter condition is wholly possible, because the range of moisture reserves from the moisture level retarding growth up to the minimum moisture capacity is sufficiently high and is for example, for the chestnut soils 800-1000 m³/hectare in the one meter layer, that is, it exceeds the usual norms of vegetative irrigation.

When the moisture reserves in the soil are maintained higher than the moisture level of growth retardation, the potato is in a condition to transport and accumulate intensively organic substance in the process of photosynthesis, which is carried on in proportion to the specific area of the leaves.

Many investigators think that a direct relationship exists between the area of the leaves and the sizes of the yield, with an increase in the maximum area of leaves up to its optimum size, 30-40 thousand m²/hectare.

Along with this, the question of how intensively the leaf surface works and how assimilates are used productively in the formation of the organs of the agriculturally usable parts of the yield (K_{khoz} according to Nichiporovich [4]) has great importance. The significance of the latter feature is also well illustrated by our data. Thus, the large leaf area of potatoes under the conditions of dry irrigated regions does not always mean a high yield of tubers (Variant 2). In Variant 3, 4, and 5, in spite of the somewhat smaller area of the leaves and decreased relationship of the weight of the leaves to the weight of the aerial parts of the plant in comparison to Variant 2, the dry weight is significantly higher and, what is especially important, the yield of tubers is almost twice as high.

In Fig. 1, the daily increments in the dry weight of potato tubers per unit of leaf surface (according to our determination of the size of effective productivity of photosynthesis) for Variant 2 and 5 for the 1956 vegetation period, and the figure for the soil temperature in the zone of tuber formation (at a depth of 15 cm) are shown. It appears that the maximum figures for the effective productivity coincide with a lag of one-two days with the minimum figures for the soil temperature, but

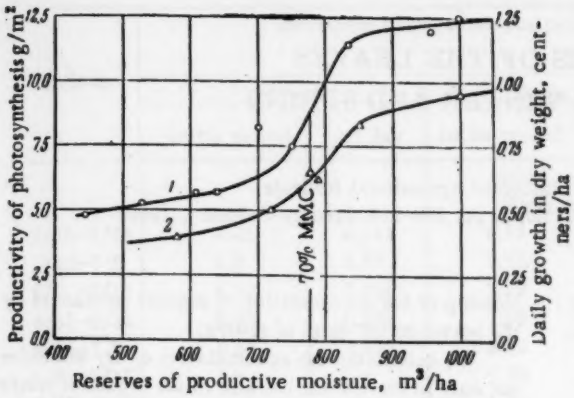

Fig. 2. The relation of the productivity of photosynthesis and the daily growth in the dry weight of the potato to the reserves of productive moisture.

1—productivity of photosynthesis;
2—daily growth in dry weight;
MMC—minimum moisture capacity.

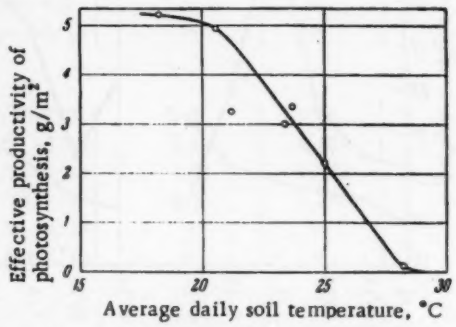

Fig. 3. The relationship of tne effective productivity of photosynthesis of potato to the average daily soil temperature in the zone of tuber formation (at a depth of 15 cm).

the minimum figures with the same lag coincide with the maximum soil temperature. The increase in the soil temperature sharply retards the flow of the products of photosynthesis into the tubers, whereupon this is registered very rapidly, not later than after a day or two. The strengthening of the flow of the products of photosynthesis begins just as rapidly with a decrease in the soil temperature. At an optimal soil temperature of 17-20°, an increased use of the products of photosynthesis in the formation of the tubers is observed. In these conditions the effective productivity of photosynthesis can exceed 5 g/m² (Fig. 3) which corresponds to a harvest of tubers of 25-30 T/hectare. With a high soil temperature, the products of photosynthesis are expended mainly in the growth of the aerial parts of the plant; the daily increment in the dry weight of the aerial parts in relation to a unit of leaf area can in these conditions reach as high as 8-10 g/m², but for the tubers is decreased to 0.5 g/m² and even down to 0.

The high productivity of photosynthesis of potatoes under conditions of irrigation in dry regions is still not an indicator of a good harvest of potatoes, because the outflow of assimilates into the tubers is often made difficult by unfavorable soil temperature. Therefore, the high yield of potato tubers in variant 5 (with winter irrigation of 2000 m³/hectare) as compared with control Variant 2 is explained not only by an increase in the photosynthesis productivity of 20-30%, but primarily by a more energetic flow of the products of photosynthesis into the tubers under conditions of decreased soil temperature (the effective productivity of photosynthesis in Variant 5 was on the average two to two-and-a-half times that of Variant 2.

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PRODUCTIVITY OF PHOTOSYNTHESIS OF THE LEAVES OF CARROT PLANTS SOWED IN THE WINTER AND SPRING

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The Department of Fruit and Vegetable Growing of the Stalingrad Agricultural Institute has carried out work for a number of years with winter sowing of vegetable crops—onion, carrot, beet, parsley, spinach, and radish. In 1956-1958 the productivity of the leaves of carrot was studied in relation to the data of sowing (plants sowed in the winter were compared with plants sowed in the spring).

Winter sowing of carrot was carried out on: November 9, in 1956; October 26, in 1957; November 10, in 1958; spring sowing, respectively on April 18, 15, and 9.

The carrots were planted in double rows in strips with 70 cm between the strips and 25 cm between the rows; after the final thinning the plants were 6 cm apart in the rows. The plot area was 25 m^2.

Shoots from the winter sowing in 1956 appeared on April 27, in 1957 on April 17, and in 1958 on April 8. Shoots from the spring planting appeared on May 8, May 3, and April 22, respectively.

The "pure productivity" of the leaves was calculated by the A. A. Nichiporovich formula,

$$A = \frac{M_2 - M_1}{(T_2 - T_1)\cdot\frac{L_1 + L_1}{2}} = g/m^2 \text{ per day}$$

where M_2-M_1 is the increment in the dry substance of the leaves and roots in the time between samples, T_2-T_1 is the number of days between samples and L_1+L_2 is the average area of the leaves for the time between samples.

Samples for analysis were taken one time in ten. We drew out 20 carrot plants of average development from which the 10 straightest were selected. The leaf area was determined with the help of millimeter paper and a planimeter and the leaves and roots were dried to a constant weight. The treatment of all samples was similar, which made it possible to compare the accumulation of dry substances of the roots and leaves for both planting dates (winter and spring) in the separate phases of growth and development of the plants.

In spite of the slight inaccuracy of this method of determining the indicator of photosynthesis (this method does not show the disposal of substances in respiration), it is nonetheless possible with its help to study the relationship of the accumulation of organic substances by the leaves to the dates of sowing.

We calculated the accumulation of dry substances not only in the leaves, but also in the organs of reserve, the roots.

From the data in the table we can see that the plant forms leaves intensively at the beginning. The main growth of the leaves for winter-sowed plants is completed by June 25. After this the growth of the roots proceeds at a more rapid pace.

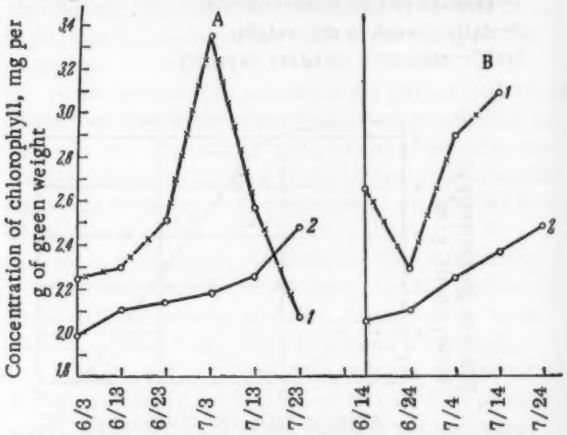

Fig. 1. The effect of dates of planting on the concentration of chlorophyll in the leaves of carrots. A—1957 experiment; B—1958 experiment; 1—winter-planted; 2—spring-planted.

The greatest accumulation of dry substances in winter-sowed plants is observed from June 15 to July 5. On the average the increment per m^2 of leaf area is 10.13 g of dry substance per day for this period. Spring-sowed plants have the highest accumulation of dry substance from July 5 to July 25. The daily average increase per m^2 of leaf surface was 9.57 g.

Winter-sowed plants give an earlier harvest than spring-sowed plants. Thus, winter-sowed carrots formed a root with an average weight of 129.01 g by July 25. Spring-sowed carrots formed a root weighing 120 g by August 15.

The data for 1957 show that the greatest increase in dry substance for winter-sowed plants was observed from June 13 to July 3. The accumulation of dry substance

TABLE. Increment in Dry Substance of Carrot Plants by Periods (in g)

Periods	Increment of dry substance per plant				Increment of dry substance per m^2 of leaf surface			Average leaf surface, cm^2
	Leaves		Roots					
	entire period	per day	entire period	per day	leaves	roots	total	
			1956 Experiment					
			Winter sowing					
5/15-5/25	0.41	0.041	0.19	0.019	5.17	2.39	7.56	79.3
5/25-6/5	0.8	0.08	0.65	0.065	5.14	4.19	9.33	155.3
6/5 -6/15	2.06	0.206	1.52	0.152	6.06	4.48	10.54	340
6/15-6/25	2.98	0.298	4.23	0.423	4.83	6.84	11.67	617
6/25-7/5	0.51	0.051	5.35	0.535	0.80	8.42	9.22	635
7/5 -7/15	–	–	2.92	0.292	–	7.03	7.03	415
7/15-7/25	–	–	1.15	0.150	–	3.06	3.06	275
			Spring sowing					
5/5 -6/5	0.40	0.040	–	–	4.84	–	4.84	82.5
6/5 -6/15	0.73	0.073	0.19	0.019	5.03	1.30	6.33	145
6/15-6/25	1.45	0.145	0.44	0.044	5.95	1.90	7.85	242
6/25-7/5	2.32	0.232	2.29	0.229	5.37	5.30	10.67	432
7/5 -7/15	0.88	0.888	3.41	0.341	4.15	5.45	9.60	625
7/15-7/25	0.52	0.052	4.87	0.487	0.81	7.62	8.43	635
7/25-8/5	–	–	2.05	0.205	–	4.19	4.19	489
8/5 -8/15	–	–	1.20	0.12	–	3.88	3.88	309
			1957 Experiment					
			Winter sowing					
5/23-6/3	0.5	0.05	0.26	0.026	5.80	3.02	8.82	86.1
6/3 -6/13	1.19	0.119	0.95	0.095	4.98	4.00	8.98	239
6/13-6/23	1.64	0.164	2.82	0.282	4.00	6.95	10.95	409
6/23-7/3	2.45	0.245	3.95	0.395	4.57	6.83	11.40	577.5
7/3 -7/13	–	–	3.15	0.315	–	5.56	5.56	566
7/13-7/23	–	–	1.78	0.178	–	4.84	4.84	367.8
			Spring sowing					
6/3 -6/13	0.21	0.021	0.11	0.011	5.06	2.78	7.84	39.5
6/13-6/23	0.45	0.045	0.42	0.042	4.00	3.73	7.73	112.5
6/23-7/3	0.96	0.096	0.10	0.11	4.41	5.12	9.53	217.3
7/3 -7/13	1.75	0.175	1.95	0.195	4.56	5.08	9.64	384
7/13-7/23	2.45	0.245	3.04	0.304	4.51	5.62	10.13	541
7/23-8/3	–	–	2.43	0.243	–	5.50	5.50	442
8/3 -8/13	–	–	1.25	0.125	–	4.61	4.61	271
8/13-8/23	–	–	0.75	0.075	–	3.84	3.84	195
			1958 Experiment					
			Winter sowing					
6/3 -6/13	0.46	0.046	0.49	0.049	4.21	4.51	8.72	109
6/13-6/23	1.08	0.108	1.10	0.11	4.48	4.56	9.04	241
6/23-7/3	2.65	0.265	3.01	0.301	4.98	5.89	10.87	511
7/3 -7/13	3.48	0.348	5.28	0.528	4.94	7.49	12.43	705
7/13-7/23	–	–	3.45	0.345	–	5.83	5.83	591
			Spring sowing					
6/3 -6/13	0.4	0.04	0.3	0.03	4.70	3.55	8.25	85
6/13-6/23	0.48	0.048	0.39	0.039	4.80	3.90	8.97	100
6/23-7/3	1.16	0.116	0.99	0.099	4.73	4.07	8.80	243
7/3 -7/13	1.59	0.159	1.97	0.197	3.61	4.58	8.19	430
7/13-7/23	2.37	0.237	3.41	0.341	3.95	5.69	9.64	599.2
7/23-8/3	2.03	0.203	3.50	0.350	3.31	5.69	9.00	615
8/3 -8/13	–	–	2.50	00.250	–	5.22	5.22	479
8/13-8/23								

per day per m^2 was 10.44 g. For spring-sowed plants the highest increase was observed from July 3 to July 13 and was 9.77 g per m^2 per day. The winter-sowed carrots had formed a root weighing 111.2 g by July 24. Spring-sowed carrots had roots weighing 100 g by August 24.

The data for 1958 show that the greatest increase in dry substance for winter-sowed plants was from June 23 to July 13; the accumulation of dry substance per m^2 of leaves was 10.78 g. The greatest increase in dry substance for spring-sowed plants was observed from July 3 to August 3; 8.91 g were accumulated per m^2. Winter-sowed carrots had roots weighing 127.8 g by July 24 and spring-sowed carrots had formed roots weighing 121.9 g by July 23.

The presence of chlorophyll in the leaves is connected to the intensity of photosynthesis. Under experimental conditions the winter-sowed carrot plants had a somewhat greater quantity of chlorophyll than the spring-sowed plants.

As a result of our investigation, data were obtained which show that winter-sowed plants have a greater increase in dry substance per m^2 of leaf surface, contain a greater quantity of chlorophyll and give earlier production than spring-sowed plants. The difference in the date of harvest was 20-30 days.

THE EFFECT OF GIBBERELLIC ACID ON THE GERMINATION OF SEEDS AND THE GROWTH OF SEEDLINGS OF FRUIT PLANTS

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The preplanting treatment of seeds with different chemical substances is used widely in agriculture with the improvement of the germination of seeds and the improvement of the growth of the plants as goals [1-4 and others]. However, the occurrence of dormancy in the seeds of many species of plants complicates this and limits the use of this method.

Numerous trials of induced sprouting of dormant seeds by means of stimulation with chemical and growth substances have not been successful [5, 6]. One is able to induce germination when the entire seed coat is removed, but the following growth is sharply suppressed for a number of plants without the action or increased temperatures on the seeds [7, 8].

Recently an article concerning the strong effect of gibberellic acid on the growth and formative processes of plants appeared in print [9]. There are indications that gibberellic acid improves the growth not only of normal, but also of dwarf plants [10, 11] and also stimulates the growth and flowering of the winter varieties and seedlings of biannuals in conditions which exclude the effect of increased temperatures [12-15]. Barton's experiments showed that gibberellic acid aids the growth of physiologically dwarfed plants that are obtained from unripe embryos of apple, Malus arnoldiana.

Besides experiments concerned with the effect on the shoots and growing plants, we also carried out experiments on the treatment of seeds with solutions of gibberellic acid. In the experiments of Kahn and others [16], the treatment with gibberellic acid of lettuce seeds, which germinate in light, increased their sprouting when grown in the dark. Donoho and Walker [17] obtained an increase in the sprouting of insufficiently stratified seeds of peach treated with a solution of gibberellic acid. There is an indication [18] that the treatment of seeds of the dwarf variety of pea sharply stimulates the later growth of the plants.

The Effect of Gibberellic Acid on the Germination of Seeds

With acceleration of the germination of seeds and improvement of the growth of seedlings as a goal, we carried out experiments in 1958 on the treatment of seeds of pit-bearing fruits with water solutions of gibberellic acid. Seeds of cherry from the 1958 harvest, seeds of different varieties of peach obtained from the Erevan Scientific-Research Institute of Horticulture, Grape Culture, and Wine Making, and also seeds of apricot and cherry from the Ukraine Institute of Garden Culture (seeds of peach, apricot and cherry from the 1957 harvest) were used for the experiments. Prior to treatment, all seeds were freed from their hard coats, and the seeds of peach and cherry also had the remaining soft layers removed from the half of the seed that had the embryo radicle.

In the experiment with cherry, freshly collected, unstratified seeds freed from the hard coat were divided into two groups. In the first group the soft coats (brown and white) were also removed from half of the seeds, and in the second group these coats were left on the seeds. The seeds of the first group (deprived of their coats) were soaked in water and seeds of the second group (with the soft coats) were soaked partially in water and partially in an 0.02% water solution of gibberellic acid. The seeds were left in the solution of gibberellic acid for two days. After treatment the seeds were placed in a Petri dish in a thermostat at 25° for germination. Observation of the germination of the seeds showed that only those seeds from which all of the coats were removed germinated, but the seeds with the soft coats, independent of the means of treatment, did not have sprouts. This is seen clearly in Fig. 1.

Thus, the treatment of seeds with an 0.02% solution of gibberellic acid does not decrease the retarding effect of the seed coat on germination.

The experiment with peach seeds began on April 26. Unstratified seeds of variety Narindzhi, Chgodi, and a mixture of various varieties were used. The experiment with apricot and cherry seeds began on May 9; both unstratified and stratified seeds of apricot variety Sorochinsk and Besseya cherry were used. The seeds of the experimental variants were soaked prior to planting for a period of 24 hours in a 0.02% solution of gibberellic acid, and the seeds of control variants in water. After treatment all seeds were planted in Petri dishes with soil and placed in the greenhouse.

As a result of the calculation of the date of the appearance of sprouts, it was found that gibberellic acid accelerated the germination of unstratified seeds of peach and cherry by three to four days, but there did not seem to be any noticeable effect in accelerating the germination of apricot and the stratified seeds of cherry. The

TABLE. The Effect of Gibberellic Acid on the Growth of Seedlings (in cm)

Species and varieties of fruit plants	Experimental Variant: Stratification	Experimental Variant: Treated with gibberellic acid	Date: May 26	Date: June 16	Date: July 4	Date: July 18	Date: August 4
Chgodi peach	Unstratified	Experiment	6,6	23,7	35,6	48,9	71,3
		Control	3,0	16,8	22,2	34,2	48,8
Narindzhi peach	Same	Experiment	8,3	25,8	33,3	45,5	61,5
		Control	4,8	22,2	32,6	47,6	62,0
Mixture of various varieties		Experiment	13,5	22,2	23,4	26,8	33,0
		Control	10,0	16,0	19,4	26,6	36,4
Apricot		Experiment	12,9	21,1	22,1	24,6	28,7
		Control	7,5	16,8	18,7	23,7	31,5
Sorochinsk	Stratified for 45 days	Experiment	13,5	29,3	38,0	45,7	59,7
		Control	12,5	28,0	42,5	48,0	65,5
Cherry	Unstratified	Experiment	- -	- -	- -	- -	7,5
		Control	- -	- -	- -	- -	5,6
Besseya	Stratified for 64 days	Experiment	- -	- -	- -	- -	9,2
		Control	- -	- -	- -	- -	8,0

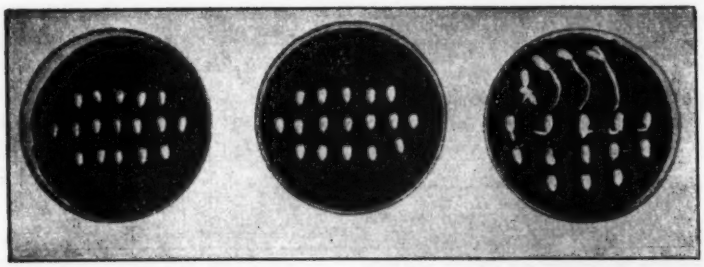

Fig. 1. The effect of gibberellic acid on the germination of cherry seeds. Left, with coats, control; center, with coats and treated with gibberellic acid; right, without coats, control.

Fig. 2. The effect of gibberellic acid on the growth of seedlings with the treatment of peach seeds. In the photo: Seedlings of Chgodi peach at two months. Left, two plants treated with gibberellic acid; right, control plants.

germination of stratified and unstratified seeds of apricot and stratified cherry seeds began simultaneously in both the control and the experimental variants. Gibberellic acid also somewhat increased the germination of peach seeds and increased the sprouting of apricot and cherry seeds.

Thus, gibberellic acid accelerates the germination of peach and cherry seeds but does not decrease the retarding effect of the soft seed covers and, therefore, cannot replace stratification. The effect of gibberellic acid on the germination of peach and cherry seeds appeared only in the case of the removal from the seeds of all seed coats. It is possible that gibberellic acid has no effect on seeds with coverings as a result of its weak penetration through the seed coverings.

The Effect of Gibberellic Acid on the Growth of Seedlings

Observations of the growth processes of peach, apricot and cherry seedlings show that they proceed in different ways in the various experimental variants. The results of the calculation of the growth processes of seedlings are presented in the Table.

The data in the table show that the growth processes in the first period of growth proceed more intensively when the seeds are treated prior to planting with a solution of gibberellic acid. Then the difference in the growth is gradually obliterated, with the exception of seedlings of Chgodi peach, in which the difference is maintained for the entire growth period of the plants. The length of the shoots of experimental plants of the variety exceeded significantly the length of the control seedlings (Fig. 2).

We must note that the effect of gibberellic acid on the growth processes of seedlings appears most sharply with the treatment of unstratified seeds and less with the treatment of stratified seeds. Thus, with treatment of stratified apricot seeds, the plants of the experimental variant, although somewhat taller than the control plants at the beginning of the experiment, had a smaller number of leaves, the leaves were lighter and the stems weaker. Subsequently, this affected negatively the growth of the experimental plants, which at the time of the last measurements began to fall behind the control plants.

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THE DEVELOPMENT OF WINTER-SPRING WHEAT IN CONNECTION WITH ITS WINTER HARDINESS

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Winter-spring wheat is relatively widely used in agricultural production in regions with a relatively mild winter, as, for example, in the south of our country, in Bulgaria, Czechoslovakia, Hungary, Yugoslavia, France, etc. Often its use is adapted to those regions where autumn sowing is carried out during the relatively short day. These wheats, while developing spikes similar to summer wheat with spring or autumn sowing, overwinter in the absence of spike formation.

The biology of the development of winter-spring wheat has been poorly developed in the literature. As the data in our experiments showed [1, 2], a retarding of the development and growth under the effect of autumn conditions and especially of the short day takes place significantly more strongly in the case of winter-spring wheats than for summer wheats, and this helps their winter hardiness.

Other authors also came to approximately the same conclusions in relation to the features of the development of winter-spring wheat [4-8]. For winter resistant varieties, as a rule more retarding of the development under the effect of autumn conditions is observed than for the poorly winter resistant varieties. Thus, with cultivation under the conditions of a shortened 12-hour day, winter-spring wheat 0109 from Bulgaria was practically not retarded in development in comparison to summer wheat, while Cheshkaya (Czech) winter-spring wheat in these conditions was retarded significantly (it did not form spikes up to the end of the vegetation period). Retarding of the development of winter-spring wheat 0109 under conditions of a 10 hour day, in comparison to summer wheat, was observed.

Furthermore, with August 11 sowing under the usual natural conditions of winter-spring wheat Novosadska 1439 (from Yugoslavia), differentiation of the point of growth was noted 11 days later than for Moskovka summer wheat, 28 days later than for winter-spring wheat 0109 (from Bulgaria), and 67 days later than for Cheshkaya (Czech) winter-spring. The same thing was also observed in relation to the intensity of growth. From this we can see that growth and development for winter resistant winter-spring wheat is retarded earlier and to a greater degree with the approach of autumn. This can be used by selection people in the preliminary appraisal of varieties and forms for winter hardiness.

The winter-spring wheats that are subjected to the continuing effect of low temperatures (about the same as those which winter wheat passes through in the stage of vernalization) in the green plant or even after the seeds have sprouted (for grains) are retarded in development and in growth under the short day conditions significantly less than those not subjected to such an effect. This also partly explains the more rapid growth and development of the winter-spring wheat after wintering in the spring in comparison to their growth and development in the autumn which often has approximately a similar length day (for example, in the southern regions).

The ability of the winter-spring plants to inhibit growth and development under short-day and poor light intensity conditions (with autumn light conditions) plays about the same role in their winter hardiness as the inhibition of growth and development for summer wheat on the long day (under normal summer light conditions).

As is well known, winter wheat which has elongated for one reason or another overwinters poorly. This can also be expected for winter-spring wheat. The relatively early planting of winter- spring wheat can result in its beginning elongation in the autumn, which in the end affects its overwintering negatively.

It follows from the data in the Table that the winter hardiness of winter-spring wheat is connected to a great extend with the date of sowing. A relatively early planting date has little effect on the winter hardiness of winter wheat, but decreases sharply the hardiness of winter-spring wheat. This is basically connected with the fact that winter-spring wheat when sowed at a relatively early date undergoes formation of the spike and elongation, whereupon for varieties of southern origin this process begins earlier and proceeds further than for varieties of relatively northern origin.

Winter-spring wheats, as well as the winter wheat, when sown in September, enter the winter with an undifferentiated point of growth in the phase of stem formation, which favorably affects its winter hardiness. Winter-spring wheats from relatively southern regions overwinter more poorly than those from northern regions; this, as was noted earlier, is connected with the fact that this characteristic is sharply inhibited in the development and growth, which under the effect of autumn conditions are less expressed.

TABLE. The Effect of the Date of Sowing on Winter Hardiness of Wheat

Variety	Percent overwintering by date of sowing			
	August 1	August 17	September 6	September 21
Moskovskaya 2453 (winter)	41	48.0	64.6	-
Cheshkaya (Czech) winter-spring	0	14.0	52.8	94.2
K-26191 (Austria) winter-spring	0	10.8	58.8	96.4
NOÉ (France) winter-spring	0	1.8	43.3	69.8
0109 (Bulgaria) winter-spring	0	0	3.5	-
Del'fi (Armenia) winter-spring	0	0	12.5	46.5
Moskovka (summer)	0	0	0	0

With obtaining a good harvest from varieties of winter-spring wheat as the goal, it is necessary to plant them on such a date in the autumn that they will enter the winter with an undifferentiated point of growth and with sufficient development (about in the phase of stem formation).

We have already noted that winter-spring plants grown from vernalized seeds develop and grow significantly more rapidly in the autumn than those winter-spring varieties developing from nonvernalized seeds, which, undoubtedly, cannot affect negatively the winter hardiness of plants grown from vernalized seeds. Thus, in our experiment with Cheshkaya (Czech) winter-spring wheat planted on October 6 from nonvernalized seeds, 186 of 343 plants overwintered, that is, 54.2%, and for this same wheat planted from vernalized seeds (about 50 days), only 24 out of 298 plants overwintered, that is, 8.0%.

The significant acceleration in the development and in the growth of winter-spring varieties can result from the extension of the natural day in the autumn period, which also affects negatively its resistance to the unfavorable conditions in the winter.

Sowing of a number of varieties on a light plot was carried out on September 21. After complete sprouting, the plants were exposed to different light conditions: continuous (500 watt lamps were used for supplementary lighting and the intensity of the supplementary light was about 4000-6000 lux), natural, and a short 9-hour day.

For winter-spring plants under the continuous lighting, differentiation of the point of growth on the spike buds and elongation were noted in the autumn; this was not so for winter wheat plants, because the stage of vernalization was not completed.

A calculation of the plants remaining in the spring showed that for winter wheat Moskovskaya 2453, 98.7% on the continuous light overwintered, 96.5% on the natural day, and 78.2% on the short day. Practically all winter-spring wheat under continuous light died. Thus, only 0.6% of the NOÉ wheat and 0.9% of the Cheshkaya winter-spring wheat survived. These same winter-spring varieties on the natural and short day overwintered completely satisfactorily, practically the same as winter wheat. Thus, on the natural day 79.4% of the NOÉ overwintered and 94.0% of the Cheshkaya winter-spring variety, and on the short day 82.2% of the Cheshkaya winter-spring variety.

The practically complete death of the winter-spring varieties under the continuous light in the autumn period is connected with the fact that they elongate in these conditions, and this has a negative effect on their hardening. In the remaining variants, the winter-spring varieties enter the winter, as does the winter wheat, with an undifferentiated point of growth, and this aids in their hardening.

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PRACTICAL APPLICATIONS

OBTAINING GIBBERELLIN AND DETERMINING ITS PHYSIOLOGICAL ACTIVITY IN CONNECTION WITH ITS APPLICATION IN PLANT GROWING

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Among the various means of chemical action on plants, biologists and plant growers have given a great deal of attention in recent times to the treatment of plants with new, biologically active substances, the gibberellins, which in many cases show a great effect on growth, development and yield of plants [1-6].

It is well known that gibberellins were found in isolated fungi of Gibberella fujikuroi Saw. (which is the sexual stage of Fusarium moniliforme Sheld.) and then were purified and identified in the form of four compounds: gibberellin A_1 ($C_{19}H_{24}O_6$), A_2 ($C_{19}H_{26}O_6$), A_3 ($C_{19}H_{22}O_6$) and A_4 ($C_{29}H_{24}O_5$). The gibberellins are related to alicyclic carboxylic acid containing a hydrogenated fluorene nucleus and possessing a high physiological activity, acting in concentrations of 1-100 parts per million parts of water (1-100 mg/liter). Among those possessing the highest activity is gibberellin A_3, which also is widely distributed under the name gibberellic acid [7-12].

Numerous experiments on the effect of gibberellins on plants carried out in Japan, U.S.A., England, the Soviet Union and in other countries, showed that they stimulate the germination of seeds, accelerate growth of stems and leaves of seedlings of annual and perennial plants, break the dormancy of buds of fruit trees, and also the dormancy of tubers and bulbs, accelerate the flowering of many annual plants, and also of seedlings of the winter forms of biannuals, result in the formation of fruits without seeds in vegetable plants, increase the dimensions of the nucleus and fruits and increase the yield of green mass of fodder grasses and other plants. Gibberellins also show a noticeable effect on the chemical composition of the yield, changing the concentrations of such substances as proteins, sugars, alkaloids, etc. In connection with such various effects of gibberellins on plants, very broad prospects have been opened for their use in plant growing with increasing the yields of vegetable and technical crops, fodder grasses and plants going into silos, forcing decorative crops, acceleration of the cultivation of forest and fruit species, etc. as goals. In recent years, both in our country and in foreign countries, a great deal of work on the study of the effect of gibberellins on the growth and yield of various agricultural plants is being done, with an examination of the possibility of their use in production conditions as a goal. [1,4,6,13-18].

In connection with this, there is the question of obtaining pure preparations of gibberellins in large quantities and the organization of their production. In connection with the synthesis of gibberellins by chemical means, and in view of the complexity of their structure, which has not been completely understood up to the present time, enzyme methods of obtaining gibberellins were developed for which a nutrient medium containing glucose, calcium hypophosphate, magnesium sulfate and ammonium chloride, is inoculated with the fungus Fusarium moniliforme Sheld. and several days after fermentation, the gibberellins are separated with the help of activated carbon using acetone or alcohol as a solvent [10].

It was in this manner that gibberellins were obtained at first in special factories in Japan (Kyowa, Fermentation Industry Co. Ltd, Tokyo, Japan); the United States (Eli Lilly and Company, Indianapolis, Ind., U.S.A.; Merck and Company, New Jersey, U.S.A.) and England (Imperial Chemical Industries Ltd., Akers Research Laboratories, Welwyn, Hertfordshire, England). Preparations of gibberellins made in these factories have obtained the widest distribution.

From the beginning of 1958, a group of microbiologists, chemists, and plant physiologists of the USSR Academy of Sciences organized work on obtaining preparations of gibberellin from the stems of the Fusarium fungus, on its chemical purity, and on an explanation of its physiological activity.

A culture of the Fusarium fungus, Fusarium sp., which was isolated from infected grape vines and which grew well both on simple synthetic and on complex organic nutrient mediums, was used. The culture on an agar medium shows good development of white mycelia with thick branching threads, and numerous microconidia are formed on the hyphs of the mycelia. The macroconidial fungus do not form, as a result of which we were unable to establish its species attachment. Under conditions of intense cultivation of cuttings in liquid, the development of the fungus takes place significantly more rapidly than under conditions of dormancy. Thus, the investigated fungus differed in its char-

acteristic from the typical stem of Fusarium moniliforme Sheld., the mycelia of which are quikly broken down into short bacillus-like cells and in general form macroconidia.

Cultures of Fusarium sp. were grown on two synthetic mediums. 1) A Stodola medium: 3.0 g NH_4Cl 3.0 g KH_2PO_4; 3.0 g $MgSO_4.7H_2O$; 30 g of sucrose (or glucose); 1 liter of tap water. 2) The other medium: 0.3 g MgCO; 0.2 g NaCl; 1.0 g KNO_3; 0.001 g FeO_4; 20 g of sucrose; 1 liter of tap water. The fungus formed an active substanc which began to be produced on the third-fourth day of growth of the culture at 25°, in both of the synthetic mediums. Separation and purification of the active substance wa carried out according to the method described by Stodola and others [10]. The culture liquid was separated from the mycelia which were mixed with the activated carbon; then the carbon was separated, washed with water, dried in air and suspended in methanol. After an hour the carbon was filtered off and the filtrate that was obtained was concentrat ed in a vacuum and subjected to further chemical purification. In the end, a crystal substance of a white color, slightly soluble in water and highly soluble in alcohol, was obtained and was called gibberellia preparation No. 1. At the same time another substance was obtained in the form of a brownish powder, highly soluble in water and called gibberellia preparation No. 2.

A comparative investigation of the physiological activity of the preparations that were obtained and gibberellin A_3 (gibberellic acid) from the U.S.A. was carried out by biological means in an experiment in which two-month old rosette plants of Rudbeckia (Rudbeckia bicolor), which were under the conditions of a short ten-hour day, were used as objects for experimentation. The plants were treated with 0.02% solutions of the substances for a period of one and one-half months with one drop of the solution containing 10 mg of the substance, placed daily in the center of the rosette and, after the appearance of the stem, in the tip of one of the upper leaves. The experiment was carried out in the spring of 1958 in the greenhouse with four repetitions; during the time of the experiment, each experimental plant received 450 mg of the substance and control plants were treated with water. The results of the experiment are given in Table 1 and in Fig. 1.

The results of investigation showed that gibberellin preparation No. 1, obtained from Fusarium sp., is not differentiated from gibberellin A_3 by its effect on the growth and generative development of plants, but gibberellin preparation No. 2 is differentiated by a weaker effect on the

Fig. 1. The effect of gibberellin preparations on the growth and development of Rudbeckia. 1—Gibberellin A_3 (gibberellic acid from U. S. A.); 2—gibberellin preparation No. 1, obtained from Fusarium sp. under laboratory conditions; 3—preparation No. 2; 4—control. (Photo, May 6, 1958).

growth of plants. Thus, it can be supposed that gibberellin preparation No. 1 is a gibberellin similar to gibberellin A_3, or gibberellic acid [19].

The development of a method of obtaining gibberellin in pilot plant conditions was started on the basis of the means of obtaining gibberellin preparation No. 1, with the accumulation of it in quantities sufficient for further broad tests on plants as a goal.

The investigation was carried out on the experimental section of the Institute of Antibiotics of the Ministry of Health of the USSR, with the Karpov Pharmaceutical Factory, in a ferment of 1000 liters with a practical charge of 600 liters.

The first charges were carried out on a Stodola medium already approved in laboratory conditions; the planted material for the growth of Fusarium sp. also was prepared on this medium in 10 liter inoculators, from which the mycelial mass was transferred to the fermenter for a stretch of three to five days of growth. The first three ferments that were carried out showed that on the first days

TABLE 1. The Effect of Gibberellic Preparations on the Growth and Development of Rudbeckia

Substance	Quantity of substance, μg	Date		Height of plant, cm	
		Elongation	Bud formation	3/7	4/25
Gibberellin A_3 (gibberellic acid)	450	2/20	3/27	16	62
Gibberellin preparation No. 1	450	2/20	2/22	13	54
Gibberellin preparation No. 2	450	2/24	3/24	3	31
Control, water	-	Rosette		-	-

TABLE 2. The Effect of Gibberellin Preparations on the Growth and Development of Rudbeckia

Substance	Quantity of substance, μg	Date		Height of plants, cm		Weight of a single plant at the end of the experiment. Sept. 18, g		
		Elongation	Bud formation	9/6	9/18	Aerial parts	Roots	Total
Gibberellin A_3 from the U.S.A.	300	8/2	8/22	54	60	65	30	95
Gibberellin preparation	300	8/3	8/23	54	55	68	40	108
Control, water	—	Rosette		—	-	39	47	86

of growth, an abundant foam is formed which reached to the cover of the fermenter, which does not decrease even when given a large quantity of foam extinguisher, vegetable oil and actodecanol. In connection with this, tests were made of the various mediums with different sources of carbon and nitrogen nutrition, as a result of which the most favorable appeared to be a medium of the following composition: 2 g $NaNO_3$; 1 g KH_2PO_4; 0.5 g KCl; 0.59 g M_9SO_4; 0.001 g $Fe_2(SO_4)_3$; 20 g of sucrose in 1 liter of water. Six fermentations were carried out on this medium, for which there was no foam formation, the given air regime and agitation were maintained up to the end of fermentation (10-11 days) the mass was quickly filtered off from the mycelia, which were made up of heavy long threads and did not break up.

The chemical purification of the culture liquid obtained after fermentation was carried out according to a scheme in which several changes in the Stodola method were introduced. The culture liquid was filtered off from the mycelium, activated mark A carbon, pulverized from a 1% mixture (by weight), was added; after one hour of agitation the carbon was filtered off by the method of elution, washed with alkaline acetone containing 3% hydrous ammonia in the relationship 1:4, and for a period of $1\frac{1}{2}$ hours was agitated at a temperature of 25-30°. Then the carbon was filtered off and the solution concentrated under a vacuum at 40° up to the disappearance of the odor of acetone and ammonia. The precipitate obtained after distillation was treated with ethyl acetone and the ethyl acetate was added with the buffer solution ($KOH+KH_2PO_4$) pH 6.5; the mixture that was obtained was acidified with 10% HCl to a pH of 3.5 and again treated with ethyl acetate until a colorless layer was obtained. The ethyl acetate solution was dried with sodium sulfate and after filtering was distilled under a vacuum until an oil-like substance was obtained. The resulting precipitate was dissolved in a small quantity of ethyl acetate and recrystallized several times from petroleum ether (boiling temperature, 60-70°). From all of the six ferments, 9.2 g of pure gibberellin preparation were obtained.

Thus, a gibberellin preparation was obtained in pilot plant conditions on the Karpov Experimental Pharmaceutical Factory [20].

The physiological activity of this preparation in comparison with gibberellin A_3 from the U.S.A. was also tested in an experiment with rosette plants of Rudbeckia under short day conditions by the same method as was used earlier, but with the difference that instead of a one and one-half month treatment, the plants were subjected to the action of the preparations for a period of one month. The experiment was established with five repetitions during the vegetation season of 1958. The results are presented in Table 2 and in Fig. 2.

The data in Table 2 and the condition of the plants in Fig. 2 suggest that the growth of plants, the rate of development and the accumulation of weight for plants treated with gibberellin A_3 and the gibberellin preparation obtained in pilot plant conditions showed the same physiological activity as gibberellin A_3, or gibberellic acid, from the U.S.A. [21].

The preparation developed and tested in this manner was sent to the Botanical Garden of the USSR Academy of Sciences where, as a result of spraying plants with a solution of the gibberellin preparation, a significant stimulation was obtained in the growth of seedlings of lilac, snowflake, hornbean and other woody bushes and also several vegetable crops [22].

With development of the method of obtaining gibberellin and the approval of the resulting preparations, there was a great deal of importance attached to the possibility of rapid determination of the activity of the substances. The applicable biological method of determining the activity of the substances on the rosettes of Rudbeckia is a method that is reliable but takes a long time. In connection with this, two other methods were developed — 1) fluorescence and 2) biotest on the leaves of corn which makes a more rapid determination of the gibberellin activity of the substances possible.

The first method is based on the fact that at the beginning, chromatographic separation of the gibberellin

on paper is carried out with the help of water saturated butyl acetate, and then its spots are developed by means of treating the paper with sulfuric acid and viewing the fluorescence in an ultraviolet light. Five ml of the culture liquid are acidified with 5% HCl to a pH of 3.5-4.0 and 1 ml of butanol is added to it, after which the mixture is agitated for 1-2 minutes and allowed to settle the layer of butanol separates and is placed on the chromatogram. In the case of a pure preparation, a batch of 1 mg is immediately dissolved in 1 ml of butanol and placed on the chromatogram. The chromatogram is dried and then after 15-16 hours is placed in a container, the lower layer of which has been saturated previously with water saturated butyl acetate. Distillation of the chromatogram is carried

Fig. 2. The effect of gibberellin preparations on the growth and development of Rudbeckia 1- Gibberellin A_3 (gibberellic acid) from U.S.A.; 2- gibberellin preparation obtained from Fusarium sp. under pilot plant conditions at the Karpov Experimental Pharmaceutical Factory; 3- preparation from yeast, Torula pulcherrima; 4- control (Photo, July 9, 1958)

out with the help of the upper layer of the water saturated butyl acetate for a period of 5-8 hours, after which it is dried and moistened with 5% sulfuric acid; after repeated drying it is developed for a period of 5 minutes at 60°. The resulting chromatogram is viewed in ultraviolet light, placed on tracing paper and the concentration of gibberellin is determined based on the area of the spot when flouresced with green light. With this the calculation is carried out according to a previously developed curve of the standard preparation of gibberellin, using gibberellin A_4 (gibberellic acid) from England [20].

The fluorescence method makes it possible to trace the accumulation of gibberellin in the various states of development of the culture fungus in the fermenters and to make a determination of the gibberellin activity of the resulting preparations. The method appears to be suited for these goals in connection with the fact that: 1) The quantity of gibberellin formed in the culture mediums of the Fusarium fungus is relatively high; 2) in the culture mediums of the Fusarium fungus in general a mixture of gibberellins is not formed, but rather gibberellin A_3 or gibberellic acid, and, in addition to this, 3) in the culture mediums of Fusarium fungus, substances whose presence would interfere with the fluorescence of gibberellin A_3 are not formed in noticeable quantities.

The biological test developed by Boyarkin and Dmitrieva, [23] and, based on the stimulating action of gibberellin on the growth of corn leaves is of more general importance.

Fig. 3. A test of the physiological activity of gibberellins by the biological method on rosettes of Rudbeckia: 1—Gibberellin A_3 (gibberellic acid from England); 2, 3—gibberellin obtained under production conditions at the Kurgansk Medical Factory; 4—control. (Photo, Sept. 1, 1959).

Five to six-day old shoots of corn are taken with the first leaf breaking through from the coleoptile, and cuttings 35 mm long are prepared from them in such a manner that 5 mm belongs to the mesocoptile and 30 mm to the leaf, including the coleoptile. The cuttings are placed in glass jars where their bases are put in the test solutions, standard solutions of gibberellin and in water (control) and are kept in a moist cabinet in the dark at a temperature of 22-25° for a period of 2-5 days. After this, measurements

of the length of the first leaf from the base of the coleoptile to the upper cut are made. The increments obtained in the test solutions are compared with the curve of growth of shoots from standard solutions of gibberellin and the concentration of gibberellin in the test solution is calculated.

A comparison of the method of biological tests with the method of determining the activity of gibberellin preparations on rosettes of Rudbeckia showed that corresponding data are obtained [24]. The method of corn leaves has the advantage that all measurements take place in a period of 3-5 days and the resulting data characterize not only the activity, but also the concentration of gibberellin. The method of using the rosettes of Rudbeckia has the advantage that it gives the characteristics for the gibberellin preparation not only of their effect on the growth processes, but also of their effect on stimulation of flowering of the plants.

After obtaining the gibberellin preparation in pilot plant conditions at the Karpov Experimental Pharmeceutical Factory and its approval by chromatographic and biological methods, the preparation of gibberellin was sent to the Kurgansk Medical Factory in Kurgansk. Here, with the cooperation of engineer G. A. Pominal'nik, the time regulation of the production of gibberellin was developed, and this included the characteristics and quality of the raw material, the specifications of the plant and equipment, the description of the technological process the normal time and distribution of the basic materials, production control, and the safety techniques. The technological process of production of gibberellin is based on technology developed in the Karpov factory and is divided into two main parts: Part 1 — the obtaining of a native solution in the fermentation works consisting of: 1) preparation of planting material, 2) cultivation of the planted mycelia in the planting apparatus, 3) fermentation of gibberellin in the fermenters, and 4) separation of the native solution from the mycelia. Part II — separation of the gibberellin and its purification, consisting of: 1) adsorption of gibberellin from the native solution, and 2) its chemical purification by the method of extraction.

On the basis of this regulation by the Kurgansk Medical Factory, the experimental production of gibberellin was organized and a significant quantity was obtained.

A test of gibberellin obtained from the Kurgansk Factory by fluorescent and biological methods (Fig. 3) showed its high physiological activity and the possibility of broad testing of it on various agricultural plants with a goal of further use in plant growing.

SUMMARY

In connection with the broad test of the effect of gibberellins on the growth and yield of different agricultural plants and with the discovery of the possibilities of using it in plant growing, a question arose on obtaining gibberellins in large quantities and organizing their production. At the present time, an enzyme method of obtaining gibberellins has been developed, on the basis of which their production was at first located in Japan, the U.S.A. and England.

From the beginning of 1958, work began in the USSR Academy of Sciences to obtain gibberellin from the stems of the Fusarium fungus, Fusarium sp. A gibberellin preparation obtained in laboratory conditions was tested by the biological method on the rosettes of Rudbeckia, and this preparation showed a high physiological activity, no worse than gibberellin A_3 (or gibberellic acid) from the U.S.A. in its effect on the growth and flowering of plants.

Further study was carried out at the Karpov Experimental Pharmaceutical Factory, where the technological process and the method of chemical purification of the gibberellin preparation were determined. A test of the preparation obtained under pilot plant conditions also showed its high physiological activity. In addition, two new methods were developed: 1) the fluorescence method and 2) the biological test on corn leaves which makes possible a more rapid determination of the gibberellin activity of culture mediums and preparations.

The last stage of investigation of the gibberellin preparation was transferred to the Kurgansk Medical Factory. The time regulation of the production of gibberellin was developed here, and included the characteristics and quality of the raw materials, the specifications for the plant and equipment, the description of the technological process, the normal time and distribution of the basic materials, production control, and safety techniques. A test of the gibberellin obtained in a large quantity in these production conditions showed its high physiological activity and the definite possibility of testing it widely on various agricultural plants, with its further use in plant growing as a goal.

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THE EFFECT OF MICROELEMENTS ON THE INCREASE OF THE VITAMIN C ACTIVITY OF TOMATOES

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Research on the importance of soil and foliar application of microelements for plant organisms in order to increase the vitamin C activity and the yields, move up the dates of ripening, and improve the taste of vegetables, has often been printed in the literature in recent times [1-8].

During the period from June, 1956, to August, 1957, we made an investigation of 230 samples of tomatoes grown in open ground and 220 samples of tomatoes grown under greenhouse conditions.

A comparison of the results of the investigation showed that greenhouse tomatoes had lower vitamin C activity than the vegetables grown under open ground conditions.

In order to increase the vitamin activity of tomatoes grown in the greenhouse, we tested means of feeding the plants with microelements. The experiment was carried out in the "Rossel'mash" factory greenhouse in which vegetables are grown in the autumn-winter-spring period for workers' dining rooms. The greenhouse vegetables serve as a source of vitamin C at the time of year when there is practically none in the stored vegetables (potato and cabbage). Therefore, we were very interested in the question of the possibility of increasing the vitamin C activity of tomatoes grown under greenhouse conditions.

The feeding of plants with the microelements was carried out monthly from the time of flowering of the tomatoes through the entire period of ripening of the fruits; one month microelements were introduced into the soil and the following month the leaves of the plants were sprayed (foliar application). The following microelements were in solutions (in 10 liters of water) for fertilizing the soil and spraying the leaves of the plants: 1.5 to 2 g of boron in the form of boric acid (H_3BO_3); 1.5 to 3 g of copper in the form of copper sulfate ($CuSO_4$); 1 g of magnesium in the form of magnesium sulfate ($MgSO_4$); 1 to 1.5 g of manganese in the form of potassium permanganate $KMnO_4$).

All microelements were introduced into the soil at the same time.

The introduction of the microelements into the soil was calculated at 10 liters of solution per ten plants; when the solution was applied by spraying, it was applied so that there appeared to be a heavy dew on the leaves of the plants.

The technical conditions of vegetable cultivation were the same as in the cultivation of tomatoes without the use of microelements, that is, the air temperature in the greenhouse at night was about 18-19°, and during the day was 23-28°, and the relative humidity was 60-70%. In order to increase yields, the following fertilizer was applied (as usual) to the soil in a solution (10 liters water): 40-80 g superphosphate; 5-10 g ammonium sulfate; 20-30 g potassium chloride.

Ten liters of the solution were also added to the fertilizer for ten plants.

Investigation of the quantity of ascorbic acid in the tomatoes grown under greenhouse conditions with and without microelements gave the following results. Tomatoes grown without the application of microelements had 18.7 mg% vitamin C (average from 95 samples), and tomatoes grown with the application of microelements had 28.03 mg% (average of 125 samples).

These data show that the vitamin C activity of tomatoes grown with the application of microelements was almost 10 mg% higher than for tomatoes not receiving microelement applications.

We investigated greenhouse tomatoes of three varieties: Krasnodarets, Pervenets and Tamanets. Data are presented in the table on the distribution of the vitamin C activity of tomatoes in relation to their variety tendencies, calculated by the method of variance statistics.

We can see from the table that when microelements are used, the tomatoes in all cases, regardless of variety, gave a significant (from 46.0 to 80.7%) increase in the vitamin C activity, as compared with tomatoes which did not have microelement applications. Variety Pervenets gave the largest percent increase in vitamin C activity, 80.7%.

We examined 100 samples of tomatoes in order to compare the vitamin C activity of variety Krasnodarets grown under the outdoor condition of the experimental plot and grown in the "Rossel'mash" greenhouse. It appeared that tomatoes from the outdoor experimental plot has 32.4 mg% vitamin C on the average; the greenhouse tomatoes with microelement application had on the average 30.22 mg% against 20.7 mg% for tomatoes grown without the application of microelements. Thus, the application of microelements significantly increased the vitamin C activity of greenhouse tomatoes, which approached the activity of tomatoes grown in the natural outdoor conditions.

TABLE. Vitamin C Activity of Different Varieties of Tomatoes Grown in the Greenhouse

Variety	Tomatoes grown				Percent increase in Vitamin C activity
	Without application of microelements		With application of microelements		
	Number of experiments	Average quantity of vitamin C, mg %	Number of experiments	Average quantity of vitamin C, mg %	
Krasnodarets	14	20,7± 2,98	42	30,2± 6,50	46,0
Pervenets	18	16,4± 4,54	38	29,6± 7,52	80,7
Tamanets	20	15,4± 2,70	20	23,4± 4,94	51,9

The work was completed under the direction of Department Director Prof. L. G. Zhitomirsk, to whom the author extends his thanks.

SUMMARY

Soil and foliar applications of microelements (boron, copper, manganese and magnesium) were carried out on growing tomatoes in the greenhouse.

As a result of the experiment, there appeared to be a significant increase in the vitamin C activity of the tomatoes grown in the greenhouse with foliar application of microelements (30.06 mg%) against the 20.4 mg% in tomatoes grown without the use of microelements.

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METHODS

A SIMPLIFIED METHOD FOR MICRORADIOAUTOGRAPHY OF PLANTS

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The method of radioautography, that is, the method of obtaining impressions of plants containing radioactive isotopes, can be used to solve a number of questions of plant nutrition having both theoretical and practical importance. With the help of this method, one can observe the spreading of various elements through the tissues of plants and the place of their greatest accumulation [1].

The method of microradioautography, which makes it possible to observe the distribution of radioactive elements in the tissues and even in the individual plant cells with the help of a microscope, is especially valuable.

A number of methods of microradioautography are known: the drop, renewable emulsion, and immersion methods [2]; however, all of these require a large expenditure of work and often lead to failure. In work with plants, the simple method of placing microscopic sections on an emulsion layer of x-ray or photographic film gives satisfactory results.

Transverse or longitudinal sections of the stem, petioles, leaves, roots or other plant organs are taken from plants that have received by some means some radioactive element, and which are now giving off β rays, are rapidly rinsed with distilled water, lightly pressed in a piece of filter paper for removal of the water, and are put on the film in a fresh form. The film with the section is covered with two to four layers of filter paper, placed in a book or between layers of paper in order to dry the sections at the time of exposure, and immediately subjected to pressure to ensure a good contact between the sections and the emulsion.

By this means an impressed picture of the section is obtained after exposure which shows the structure of the given plant organ, and under it in the layer of emulsion is the grain structure of the reduced silver showing the part of the section in which most of the radioactive element is located. Prior to development, the film is washed in water for a period of several minutes in order to remove the sections attached to the emulsion. Only after the removal of the sections can the film be developed.

When working with a new object, it is necessary to make microphotographs of those cells taken for radioautography each time. In addition to this, as a control to show which changes in the film are caused by plant sections in the absence of radioactivity, one must take similar sections from plants growing under completely identical conditions, but which have not received radioactive isotopes. Blackening of the film is usually not observed at all in these conditions, and only the mechanical action of the sections appear on the film emulsion.

The prints of the radioautographs obtained after photo enlarging without the use of a microscope usually give an idea of the tissues of the plants. Prints of cross sections of the tomato stem which received P^{32} for a period of 52 hours from a solution containing 9.3 μC per 100 ml are shown in Fig. 1.

Tomatoes were grown for one month without the application of radioactive isotopes. When P^{32} and Ca^{45} were applied, they were transferred to glass vegetation containers one-third filled with a normal nutrient solution, into which the greater part of the root system was placed. On a stand above the level of the nutrient solution we fixed a small container with a solution of radioactive isotope into which we placed one of the root strands of the plant.

The cuttings were made at 2, 7, 11 and 15 cm from the tips and were not rinsed in distilled water prior to being placed on the film. The quantity of radioactive phosphorus in the plants was greater the closer the section was to the tip.

In the lower sections, phosphorus is localized in the conducting tissues, but in the upper parts, (the younger parts of the plants,) it passes into the parenchyma cells of the pith and into the exterior, bark part of the stem. Similar sections of plants that had received P^{32} for a period of two hours only showed that during this time phosphorus is distributed through the stem more equally, with some predominance in the lower part of the stem.

A cross section through the root of the same tomato plant made below the root thread which was placed in the solution containing P^{32} shows that an intensive movement of phosphorus into the ends of the roots takes place (Fig. 2). A local concentration of phosphorus is observed in the individual rootlets. This can be explained either by the presence in these points of energetically dividing cells prior to the appearance of lateral roots or by the presence here of compounds connected with phosporus, primarily iron.

The distribution of Ca^{45} in the stem of these same tomato plants which received a radioactive Ca isotope for a period of 50 hours from a solution with an activity of 5.9 μC per 100 ml, also showed the concentration of Ca^{45} in younger parts of the stem (Fig. 3). However, a significant quantity of calcium also remains in the older parts of

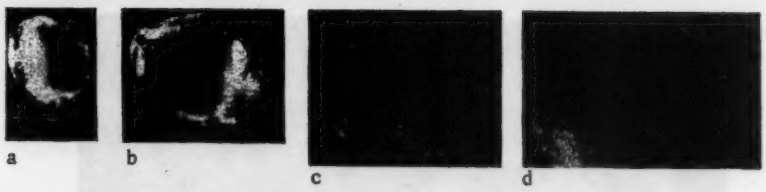


Fig. 1. Distribution of P^{32} in the stem of tomatoes 52 hours after introduction of radioactive phosphorus. Distance from the tip: a) 2 cm; b) 7 cm; c) 11 cm; d) 15 cm.

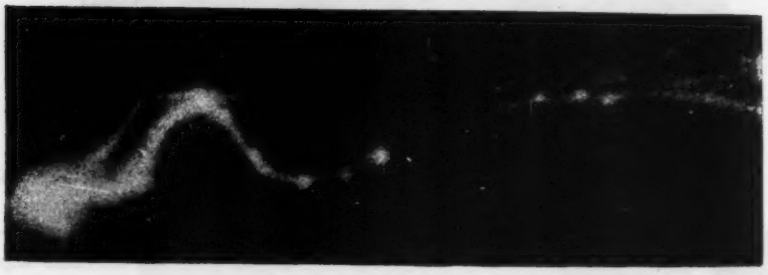


Fig. 2. Cross section of the main root and lateral roots below the strands of the roots receiving P^{32}.

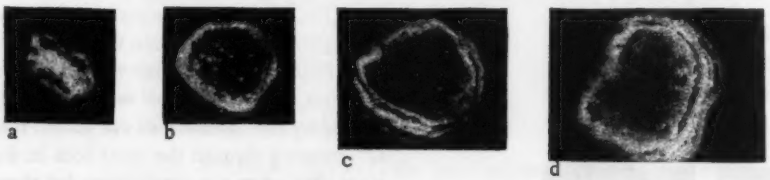


Fig. 3. Distribution of Ca^{45} in the stem of tomatoes 50 hours after the introduction of radioactive calcium. Distance from the tip: a) 2 cm; b) 7 cm; c) 11 cm; d) 15 cm.

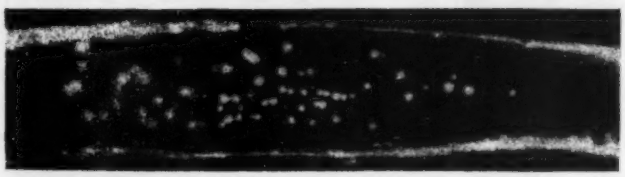


Fig. 4. Longitudinal section of the stem of tomato receiving Ca^{45}.

the stem. Therefore, the sharp gradient of concentration of phosphorus in the stem was not found in the case of calcium. It is generally known that calcium is always accumulated in the older organs of the plant, particularly in the leaves; the stronger movement of marked calcium into the younger parts of the plant as observed in this case does not contradict this. Calcium obviously is needed by the young, actively growing organs of plants and it moves there most intensively, but insofar as calcium is not reused in plants, the calcium accumulates in organs in proportion to their age.

In order to compare the concentration of radioactive elements in the various parts of plants, it is necessary to have a similar thickness for all sections, obtainable simply with the help of a hand microtome.

The thickness of the sections for radioautography, as shown in Figs. 1 and 3, is 0.25 mm.

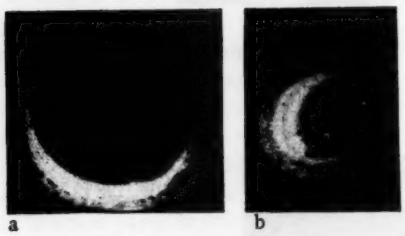
Attention must be turned to the inequality in the distribution of calcium. Clear points are seen on the radioautographs of all sections, which, apparently, are nodes of calcium oxalate; these points are seen still better under the microscope on a radioautograph of a longitudinal section of the tomato stem (Fig. 4); they are most common in the vascular part of the stem, but are also found in the pith.

The distribution of calcium in the rootlets and in the central root that received Ca^{45} directly from the solution

Fig. 5. Radioautographs of roots receiving Ca^{45}; center, longitudinal section of the main root of tomato.

a b

Fig. 6. Cross sections of the stem (a) and root (b) of mountain ash which received $Na_2C^{14}O_3$ through the soil.



a b

Fig. 7. Cross sections of stem (a) and roots (b) of mountain ash which received $C^{14}O_2$ through the leaves.

was not equal. Along with the higher and lower concentration of Ca^{45}, there were spots that contained no radioactive calcium. This is seen especially clearly on the main root (Fig. 5) where the dark central part and, in addition, separate round spots, are visible. This interesting effect is still not explained. Similar dark spots are also seen on the cross sections of the stem of tomatoes receiving Ca^{45} (Fig. 3.)

The proposed method of microradioautography can be used with success for studying the paths of the movement in plants of the elements of mineral nutrition and assimilates, and for determining the rate of this movement under various conditions.

When feeding plants with radioactive carbon dioxide through the leaves or roots, we saw that C^{14} moves in different paths in these conditions. Three-year-old seedlings of mountain ash grown in the Polar-Alpine Botanical Garden on the Kola Peninsula were given feeding with a solution of radioactive $Na_2C^{14}O_3$ in the soil close to the root system. Ten days after application, cross sections were made of the one-year-old shoots of the stem and central root (Fig. 6). Simultaneously, other mountain ash plants were given carbon dioxide through the leaves over a period of three hours; in this variant cross sections of the stem and root were made after seven days (Fig. 7). As can be judged by the intensity of the prints, there was much less C^{14} entering through the roots both in the root and in the stem than when the plants were fed through the leaves. When fed through the roots, C^{14} moved out equally through the entire stem and root: Three concentrated circles containing C^{14} were seen in the root and only two were visible in the stem, and an external circle located in the bark part of the stem contained more C^{14} than the internal part. Apparently the accumulation of C^{14} in the external part of the stem shows up not from the movement of C^{14} compounds around the periphery of the stem, but is a result of assimilation of $C^{14}O_2$ with the help of the chlorophyll that is located here, as was observed in the experiments of A. L. Kursanov with beans [3].

When feeding plants carbon dioxide through the leaves, carbon compounds move downward through the stem only from the side on which the leaves receiving $C^{14}O_2$ are located. From the zone impregnated with C^{14}, rays lead out towards the center of the stem, showing that the compounds containing C^{14} also move in a radical direction. These rays are seen especially clearly on a microphotograph (Fig. 8) of a small part of the radioautography of the stem section, the photograph of which is shown in Fig. 7.

The microphotograph is made from the film, that is, from the negative image; therefore, the places containing much C^{14} appear to be black. When this image is compared with microphotographs of the cross section of the stem of mountain ash (Fig. 9), one can reach the conclu-

Fig. 8. Microphotograph from radioautograph of the stem of mountain ash shown in Fig. 7.

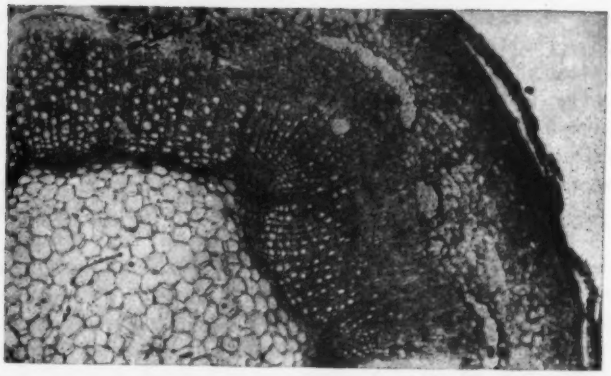


Fig. 9. Microphotograph of the cross section of the stem of mountain ash.

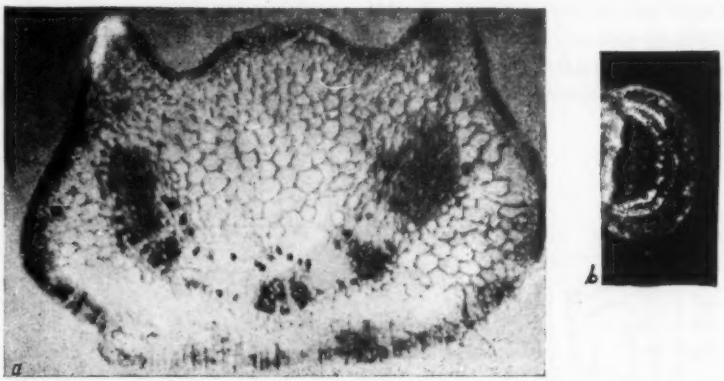


Fig. 10. Microphotograph of a cross section of the petiole of a potato leaf (a) and radioautograph of sections of a similar petiole attached to leaves receiving $C^{14}O_2$ (b).

sion that the entire phloem and part of the primary xylem are filled with carbon compounds and that a significant part of them move into the outer bark and only the dense cells of the fibrous inner bark do not contain C^{14} compounds.

A microphotograph of a cross section of the pitiole of a potato leaf is shown in Fig. 10 along with a radioautograph of a similar section of the leaf petiole located below the leaf receiving radioactive carbon dioxide.

The petiole was cut 23 hours after the plant was fed with $C^{14}O_2$.

Similar investigations of the stem of potatoes 48 hours after the plants were fed with carbon dioxide show that compounds with radioactive carbon are concentrated mainly in the xylem. The section was not rinsed in distilled water prior to being placed on the film and as a result dark spots from the sap flowing from the vessels during preparation of the sections are seen here. A microphotograph of a longitudinal section also showed the presence of radioactive compounds in the large vessels of the xylem.

The examples that are presented show that the proposed simplified method of radioautography of microtome sections of various plant organs can be used for mass investigations in resolving questions of plant nutrition.

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A SPRAYER FOR FINE SPRAYING OF LIQUIDS AT LOW PRESSURE AND LOW OUTPUT

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A number of devices are used at the present time for fine spraying of liquids in agricultural production. Some of these devices, as is well known, are atomizers.

Many authors have proposed construction of sprayers which work both directly from the pressure of the liquid itself and from the introduction into the sprayer of gas or air from a compressor. In accordance with the conditions under which they are used, they have various arrangements and corresponding parameters [1].

We were able to examine and try out several types of sprayers when building one for our purposes, using conditional construction. It was observed while studying them that either they are very complex in construction and expensive to make and use, or they give a coarse spray. In addition, most of them work at relatively high pressures and discharge a significant quantity of liquid. We developed the type of sprayer (Fig. 1) which is described in the present article when resolving the problem of a fine spray of water with low pressure and low output.

The sprayer consists of two parts — the basic part, A, and the head, B. The material from which the sprayer is constructed can differ depending on the conditions of its use. The rear part of the base of sprayer I is screwed into any liquid supply system (pipe or hose) by a special device with the help of stay II. In order to strengthen the head of the sprayer, a thread III is cut into the base of the sprayer. In the shaft of the base of the sprayer, an internal hole IV and a cross hole V are drilled for the input of liquid into the chamber XII (Fig. 1c), which is formed when the head of the sprayer is screwed into its base. A cone VII is turned into the forward part of the sprayer and two canals VI are cut under the corner in the angle of the cone for movement of liquid from chamber XII into chamber XIII.

A thread VIII in the head of the sprayer is formed for screwing it onto the base, and also chamber XII is bored out. In addition, the cone shaped chamber X is bored out in the head, in which the cone VII of the base of the sprayer fits loosely (as is seen in scheme C). The nozzle of the sprayer XI is bored out in the upper part of the cone shaped notch.

The dimensions of the parts of the sprayer are shown on the diagrams which on necessity can be changed both by increasing them and by decreasing them. The principle of the work of the sprayer is as follows. The spray liquid enters from the internal cavity of the base of the sprayer IV into the opening V (see scheme C). From the latter it moves under pressure into the circular chamber XII formed by the walls of the head and the base of the sprayer. After this, the liquid moves into the cone shaped opening VI and enters into the chamber by a spiral movement of the liquid XIII. Revolving in the chamber, it moves under pressure into the nozzle XI and, moving out from it, is sprayed.

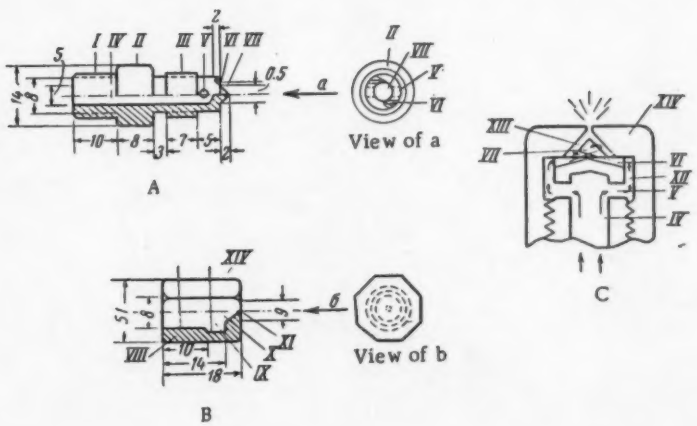

Fig. 1. Spraying device and schematic representation of its operation.

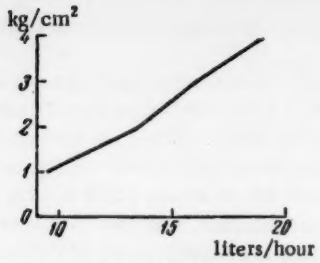

Fig. 2. Curve of the water output of the sprayer under various pressures.

The sprayer has the following output of liquid in relation to the pressure used, with the clearance given in the diagram (Fig. 2).

The size of the spray jet is: 75 cm in height and 55 cm in diameter at 4 atmospheres; 75 cm and 40 cm at 2 atmospheres; and 70 cm and 35 cm at 1 atmosphere.

In addition, the sprayer was tested at a pressure of 4 atmospheres with a nozzle 1.6 mm in diameter. The output of water in this case was 21.5 liters/hour. The advantages of this sprayer in preference to others are: 1) It consists of only two parts; 2) it is simple to construct; 3) it has small dimensions; 4) it is cleaned rapidly and well when plugged; 5) it begins to operate at low pressures; 6) it gives a fine spray; 7) a small quantity of liquid is put out.

The sprayer can be used in various conditioning installations, in sprayers, spray rooms, etc., and especially in the application of small doses of highly active substances on plants.

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